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Review

Evolution of the life cycle in land plants

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Abstract All sexually reproducing eukaryotes have a life cycle consisting of a haploid and a diploid phase, marked by meiosis and syngamy (fertilization). Each phase is adapted to certain environmental conditions. In land plants, the recently reconstructed phylogeny indicates that the life cycle has evolved from a condition with a dominant free-living haploid gametophyte to one with a dominant free-living diploid sporophyte. The latter condition allows plants to produce more genotypic diversity by harnessing the diversity-generating power of meiosis and fertilization, and is selectively favored as more solar energy is fixed and fed into the biosystem on earth and the environment becomes more heterogeneous entropically. Liverworts occupy an important position for understanding the origin of the diploid generation in the life cycle of land plants. Hornworts and lycophytes represent critical extant transitional groups in the change from the gametophyte to the sporophyte as the independent free-living generation. Seed plants, with the most elaborate sporophyte and the most reduced gametophyte (except the megagametophyte in many gymnosperms), have the best developed sexual reproduction system that can be matched only by mammals among eukaryotes: an ancient and stable sex determination mechanism (heterospory) that enhances outcrossing, a highly bimodal and skewed distribution of sperm and egg numbers, a male-driven mutation system, female specialization in mutation selection and nourishment of the offspring, and well developed internal fertilization. The study of evolution of the land plant life cycle requires a multidisciplinary approach that considers morphology, development, genetics, phylogeny, ecology, and evolution in an integrated fashion, and will deepen our understanding of plant evolution.
Key words diploidy, evolution, haploidy, land plants, life cycle.

The life cycle of sexually reproducing eukaryotes consists of a haploid (1N) and a diploid (2N) phase. This basic pattern of life cycle likely occurs throughout eukaryotes, as syngamy (fertilization) and meiosis probably evolved during eukaryogenesis (Egel & Penny, 2007; Gross & Bhattacharya, 2010). During approximately two billion years of eukaryotic evolution (Knoll et al., 2006; Payne et al., 2009), these two phases have been targets of natural selection and consequently have adopted the different size, morphology, physiology, and temporal length seen in today's diverse eukaryotes.

In many "protists", both phases are unicellular and free-living (e.g., *Chlamydomonas* (Raven et al., 2005)). In other eukaryotes, the haploid phase becomes multicellular, sometimes with tissue differentiation and organogenesis, and the diploid phase is a single-celled zygote (e.g., *Chara* (van den Hoek et al., 1995)). In still other eukaryotes, both haploid and diploid phases become multicellular (e.g., *Selaginella* (Schulz et al., 2010)). Finally, many derived eukaryotes spend their haploid phase in the single cell (gamete) stage whereas their diploid phase becomes multicellular (e.g., mammals). Besides size, a further complicating factor in shaping life cycle diversity is whether one phase is nutritionally dependent on the other. In large photosynthetic eukaryotic lineages such as red algae, brown algae, and viridiplants (green algae and embryophytes), more than one of these life cycle types can be found (Bold & Wynne, 1985; Bell, 1994; Graham & Wilcox, 2000a; Coelho et al., 2007; McManus & Qiu, 2008) (Fig. 1), probably because of the diverse niches occupied by members of these clades.

In this report, we first briefly review the history of the study of life cycle and its evolution in land plants (embryophytes). This will be followed by a discussion of adaptive advantages of haploidy and diploidy and environmental conditions that favor each life cycle strategy. We will then trace the evolutionary trend of the life cycle in land plants using an updated phylogeny. Finally, we will outline future directions of research that are likely to help us gain insights into mechanistic aspects of life cycle development and evolution in land plants.

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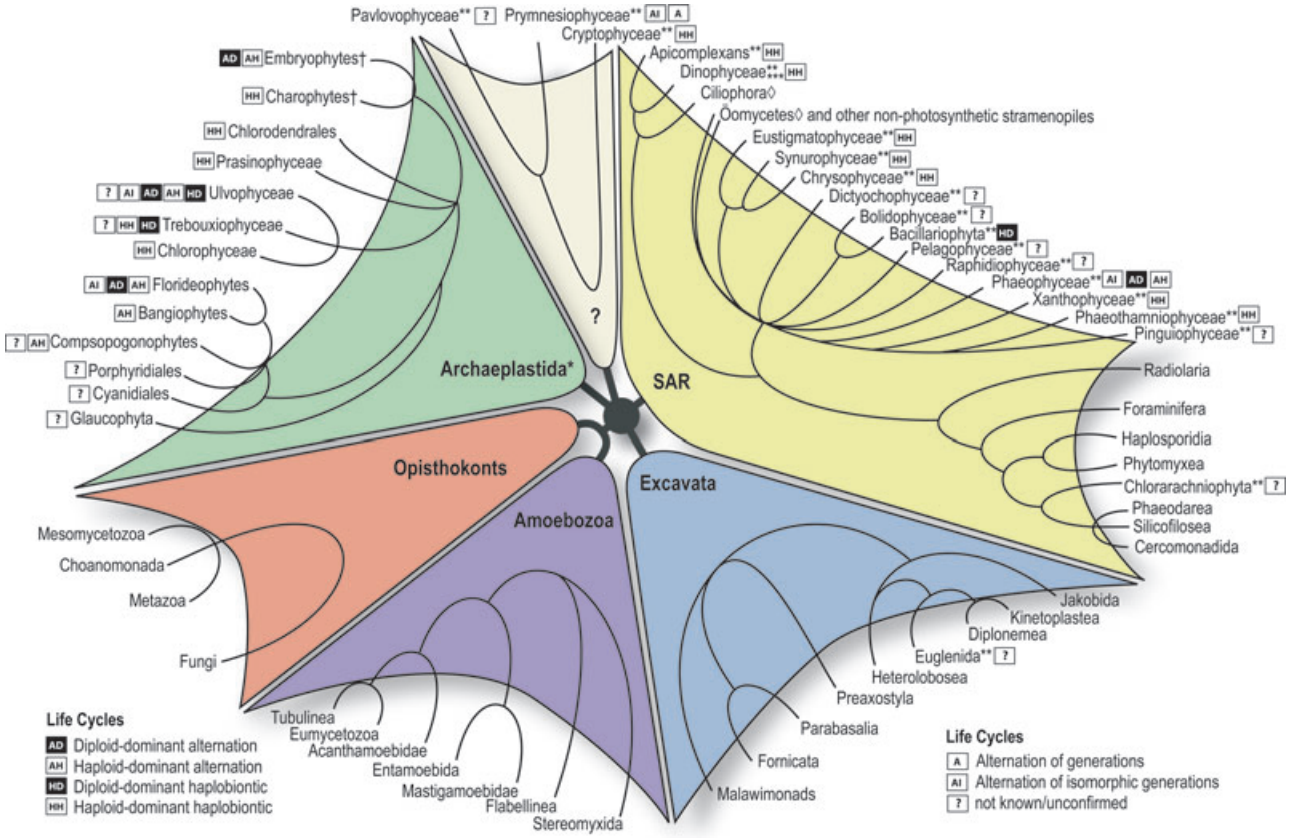

Fig. 1. Life cycle diversity in photosynthetic eukaryotes, mapped on a phylogeny of eukaryotes that depicts several "supergroups". Although not indicated, some groups are paraphyletic. Archaeplastida may not be a monophyletic group according to a recent study (Parfrey et al., 2010), but is retained here for illustrative purposes. The phylogeny is based on recent molecular studies of relationships among major lineages of eukaryotes (Kawachi et al., 2002; Baldauf, 2003, 2008; Andersen, 2004; Baldauf et al., 2004; Lewis & McCourt, 2004; Saunders & Hommersand, 2004; Adl et al., 2005; Andersson et al., 2005; Keeling et al., 2005; Simpson et al., 2006; Hackett et al., 2007; Rodriguez-Ezpeleta et al., 2007; Parfrey et al., 2010). The life cycle information was obtained from various sources (Bold & Wynne, 1985; Bell, 1994; van den Hoek et al., 1995; Kondrashov, 1997; Graham & Wilcox, 2000a; Houdan et al., 2004). †According to one study (Adl et al., 2005), the embryophyte lineage (also known as Plantae) is grouped within the Charophyta (Chloroplastida), as a member of the subdivision Streptophytina. Depicted here is a lineage labeled charophytic algae, representing all of the algal lineages within the Charophyta, and embryophytes are illustrated separately for the purpose of this review. *Groups with plastids of primary endosymbiotic origins. **Groups with plastids of secondary endosymbiotic origins. ***Groups with plastids of tertiary endosymbiotic origins. ◊Groups with plastids lost. ?Three lineages that do not group within any of the other "supergroups". SAR, stramenopiles, alveolates, Rhizaria.

1 Brief history

More than 150 years ago, it was discovered that all land plants shared a life cycle with two alternate multicellular generations (Hofmeister, 1851, 1862). This discovery was made on the backdrop of naturalists describing life histories of plants and animals (Sars, 1837, 1840; Steenstrup, 1842, 1845) (also see (Rinard, 1981)). One generation, the sporophytic one, was soon recognized to be phylogenetically newly arisen and deemed to be *antithetic* to the original, gametophytic, one, as it shows a different morphology, carries out different functions, and obeys different growth laws under environmental selection pressure (Čelakovsky, 1874). Even though the entire process of sexual reproduction was not completely understood at the time, the role of sexual reproduction in formation of the sporophyte to compensate for the defective effect of some traits inherited over time, now understood to be due to accumulated deleterious mutations (Kondrashov, 1988), was astutely recognized by Čelakovsky (1874). For sexual reproduction, only fertilization, and not meiosis, was known, and discovery of the latter happened almost 10 years later (Van Beneden, 1883). In opposition to the antithetic hypothesis, supporters of the homologous hypothesis contended that the two generations were homologous to each other and that the nutritionally dependent diploid generation in bryophytes (a paraphyletic group including liverworts, mosses, and hornworts) represented an evolutionary reduction that occurred when algal

ancestors with an alternation of isomorphic generations colonized land (Pringsheim, 1878). Discovery of doubling of the chromosome number in the sporophyte as opposed to the chromosome number in the gametophyte was a watershed event in the study of alternation of generations in plants (Guignard, 1885; Overton, 1893; Strasburger, 1894), and revealed the previously unrecognized difference between the two generations at the cytological level. Until then, the exact nature of and differences between the two generations had been contentiously debated by adherents of the two hypotheses. In fact, such was the confused state of affairs that alternation of generations of plants, algae, and fungi was mixed up with metamorphosis in animal development, which does not involve any chromosome number change (Rinard, 1981). Despite recognition that the sporophyte represents an evolutionarily new generation (Čelakovsky, 1874), it was only 25 years later that a mechanism was proposed to explain how it came about: the delay of meiosis (Bower, 1908). However, an unsatisfactory explanation was given by Bower for why delay of meiosis was favored when green algal ancestors of plants colonized land, that is, to produce a large number of spores to ensure enough fertilization events in a water-deficient environment. This was because floridean red algae, which are all marine, also evolved elaborate multicellular carposporophyte and tetrasporophyte generations (Yamanouchi, 1906a, 1906b; Svedelius, 1927) (Fig. 1). Svedelius (1927) cited the fact that meiosis allowed production of genetically variable gametes through independent assortment as the reason why its delay was selected for during evolution of the sporophyte generation in fungi, algae, and plants, because a large multicellular sporophyte could afford a large number of meiocytes. Two points should be added here, one being that the author did not mention recombination, the importance of which was suggested decades later (Grant, 1963), and the other being that the author was not able to explain why the red algae needed to delay meiosis in an aquatic environment. This latter point was made somewhat clearer 50 years later, as delay of meiosis was proposed to compensate for loss of the flagella in the male gamete (Searles, 1980). A further explanation is suggested here: union of non-motile gametes facilitated by ocean currents is beneficial in preventing inbreeding (outcrossing might have contributed to the large diversity of red algae in modern oceans). Besides the red algal life history, another piece of evidence that is less consistent with Bower's (1908, 1935) idea than the genetic explanation comes from the relative sequence of mitosis and meiosis during sporogenesis in land plants. In all land plants many mitotic divisions occur before, not after, meiosis, which suggests that it was the genetic diversity, rather than the mere number of spores (and therefore gametes), that was the target of natural selection. If the latter were true, one would expect a random distribution of mitosis before or after meiosis in red algae and land plants. Apart from this weakness, Bower's (1908, 1935) work brought the antithetic hypothesis from its prototype developed by Čelakovsky (1874) to its modern form, which explains the origin and evolution of land plants as well as evolution of the life cycle in land plants.

2 Haploidy or diploidy, which is better?

Because derived lineages of eukaryotes, in particular those large, multicellular, and terrestrial clades such as plants, animals, and fungi, exhibit a life cycle with a dominant diploid (or dikaryotic in the case of fungi) generation (Coelho et al., 2007; McManus & Qiu, 2008) (Fig. 1), it is generally believed that diploidy has been favored during evolution of eukaryotes (Perrot et al., 1991). However, the fundamental question of whether haploidy or diploidy is advantageous has not been definitively answered (Kondrashov & Crow, 1991). We emphasize here that unless the environment is considered, this is a moot question. Below we will discuss relative advantages of haploidy and diploidy under different selection pressures, and also describe some environmental conditions under which each ploidy level is favored.

2.1 Haploidy

It is widely assumed that life originated as haploid organisms and existed in that condition for over a billion years, as all prokaryotes are effectively haploid. Further, eukaryotes existed with a haploid generation-dominated life cycle for quite some time before a diploid generation-dominated life cycle appeared. This pattern is probably most evident in photosynthetic eukaryotes, where overlaying diverse life cycles of major lineages onto the phylogeny (Adl et al., 2005; Keeling et al., 2005; Baldauf, 2008; Parfrey et al., 2010) clearly shows an evolutionary trend of the dominant generation proceeding from haploidy to diploidy, but the haploid generation-dominated life cycle is still found in many, particularly basal, lineages of different algal groups (McManus & Qiu, 2008) (Fig. 1). What should be emphasized in the context of this article is that bryophytes, with a dominant haploid generation, may have dominated the terrestrial vegetation for perhaps as long as one hundred million years before vascular plants evolved (Strother et al., 2004), and even today are still very successful in certain niches (Schuster, 1966; Crum &

Anderson, 1981). Hence, the question is not whether haploidy has any adaptive advantage, but rather in what environment (Mable & Otto, 1998).

Several authors have suggested advantages of haploidy from various perspectives. One of the first observations was that cells with less DNA are smaller, grow faster, and have a shorter cell cycle. Organisms of this type are under *r*-selection (Cavalier-Smith, 1978), which occurs in populations that are relatively free of density-dependent restraint (MacArthur & Wilson, 1967). This idea seems to explain why most unicellular eukaryotes (protists) live in aquatic environments where nutrient (CO_2, O_2, organic carbon, and mineral nutrients) supply is limited and spend most of their life cycle in the haploid stage. From an ecological perspective, Lewis (1985) proposed a "nutrient-sparing hypothesis", which states that the higher surface area:volume ratio in haploids facilitates greater transport of nutrients and thus confers an advantage in nutrient-limited environments. In addition, the nutrients saved by maintaining half as much DNA would allow the organism to grow more quickly. This effect would be magnified in autotrophs, where the collection of energy is dependent on the collection of nutrients, so nutrients (such as phosphorous and nitrogen, which are needed to duplicate DNA) may be the limiting factor in cell growth (Lewis, 1985). Bryophytes are pioneering plants both historically and in today's environments, and they grow mostly in nutrient-limited habitats (Schuster, 1966; Crum & Anderson, 1981; Schuster, 1992a, 1992b). This ecological hypothesis is largely consistent with empirical observation.

Genetically, a haploid dominant generation offers a major advantage of eliminating deleterious mutations from the genome because all genes in the genome are under environmental selection during the haploid generation (Otto & Marks, 1996). Experimental tests in *Saccharomyces cerevisiae* have shown that haploid populations adapt faster than diploid ones when population size is large (Zeyl et al., 2003). This advantage partly explains why during the colonization of land by plants, bryophytes, which have a dominant haploid generation, occupy transitional niches better than most vascular plants, which have a dominant diploid generation. Nevertheless, eliminating deleterious alleles from the genome during the haploid generation is a double-edged sword, and it may have been responsible for the small genome size in bryophytes (Leitch et al., 2005), which naturally limits their evolutionary potential. After all, a deleterious allele may turn out to be beneficial when time and environment change.

Another potential advantage of haploidy, in the context of this article, is an extension of the benefit of the size limitation imposed on cell and organism by haploidy (Cavalier-Smith, 1978), which has a particular implication in early land plants. Because fertilization in bryophytes and pteridophytes (also a paraphyletic group that includes lycophytes and monilophytes) is external and dependent on environmental water, the small size of their gametophyte offers a major advantage to sexual reproduction in suitable environments. All these basal land plants have flagellated sperm, which fertilizes the egg by swimming through a layer of water on the plant surface. When plant size is small and the surface area:volume ratio is large, it is relatively easy to form the required water layer, whether the water comes from raindrops or condensation, the latter being more easily realized in the narrow layer of atmosphere right above the ground because of the unique microclimate (McManus & Qiu, 2010). This particular piece of organismal natural history reflects an interplay of general rules of morphology, development, geometry, and physics and indicates how different selection pressures interact to define a particular niche space.

2.2 Diploidy

An often-cited advantage of diploidy is the ability of heterozygous diploids to "mask" recessive alleles through interaction of dominant and recessive alleles (Crow & Kimura, 1965). The evolutionary dynamics of carrying a silenced mutational load fits with the notion of diploids being better suited for heterogeneous environments than haploids. Silenced alleles in diploids also have an advantage in their ability to mutate relatively free of selective pressure, giving diploids a kind of genetic bank of possibly beneficial alleles to be selected according to environmental circumstances. They can then become new beneficial genes through gene duplication, whereas in haploids gene duplication would have to occur before one copy of a gene could mutate through a deleterious intermediate stage to neofunctionalize (Lewis & Wolpert, 1979; Mable & Otto, 1998). For this reason, beneficial mutations may be fixed faster in diploids, a conjecture that has received some experimental support (Paquin & Adams, 1983). This diploid masking ability may be especially advantageous for large, multicellular organisms, which undergo many mitotic divisions and thus have a high likelihood of carrying deleterious recessive somatic mutations in some cells (Orr, 1995). It is also likely to be beneficial to organisms living in more mutagenic environments, such as under increased UV light, for the same reason. This is especially important for land plants, which made the transition from haploid to diploid dominance as they became better adapted to the terrestrial environment (McManus & Qiu, 2008). In these respects, which are

like many other selection pressures on life cycle evolution, diploidy is favored in multicellular organisms that live in terrestrial habitats.

The second advantage of diploidy, which was first suggested almost a century ago (Svedelius, 1927) but was rarely mentioned in the literature until recently (Grant, 1963; Searles, 1980; McManus & Qiu, 2008; Qiu, 2008), is that it allows the potential of the meiosis–syngamy reproductive system of eukaryotes for producing genetic diversity to be fully realized through recombination, independent assortment, and random union of gametes (or outcrossing). Only in a diploid cell can meiosis occur, and when the diploid organism becomes multicellular and the number of meiocytes increases, this potential is greatly increased (Svedelius, 1927; Grant, 1963). It is for this reason that the origin of eukaryotic sex has been deemed to be one of the major transitions in evolution (Maynard Smith & Szathmary, 1995). Diploidy is a necessary precondition for evolution of complex heterospory-mediated reproductive systems in derived lineages of land plants, which are discussed later in this report.

The third advantage of diploidy, related to its coupled emergence with meiosis during the origin of eukaryotes, is that it allows genome duplication through abnormal meiotic cell division. Genome duplication is now recognized as a major mechanism for generating evolutionary novelties (Ohno, 1970). When the diploid phase of an organism becomes multicellular and a large number of meiocytes can be afforded, the chance of genome duplication is significantly increased. In land plants, genome size increased by leaps and bounds after the diploid generation became dominant in the life cycle (Leitch et al., 2005). The size and complexity of plants also increased dramatically. In this context, it is worth mentioning that there is simply no large exclusively haploid organism.

The fourth advantage of diploidy, which is not completely understood, concerns heterosis (or hybrid vigor) (Lewis & Wolpert, 1979; Maynard Smith & Szathmary, 1995). Heterosis involves interaction of alleles from somewhat diverged parents and can occur only in the diploid phase of an organism. Given the widespread existence of polymorphism in nature and incomplete reproductive isolation of many species, heterosis might play a larger than expected role in intraspecific and interspecific competition.

Finally, diploidy may confer adaptive advantages to the host in evolution of the immune system as biotic interactions become more complex with continuous increase of biodiversity. Land plants took on an increasing pathogen load as they began to grow larger and live longer in an environment shared by more biotic partners, necessitating a stronger immune system capable of defending against more bacterial, fungal, and other parasites and distinguishing pathogens from beneficial symbionts, which seem to have played an important role in colonization of land by plants (Wang et al., 2010). Diploidy again confers an advantage here, as heterozygosity in genes encoding recognition proteins allows the organism to recognize a wider range of pathogens. Plant innate immune systems recognize pathogens with a two-pronged approach: pattern recognition receptors (PRRs) recognize structural elements of pathogens, and resistance (R) proteins recognize specific effector molecules used by pathogens to disrupt plant cell processes (Jones & Dangl, 2006). The PRRs specifically bind to highly conserved pathogen-associated molecular patterns, which are essential structural features of the pathogen such as flagellin or peptidoglycan (Zipfel, 2008). The R proteins detect either pathogenic effector molecules themselves or self-molecules modified by effectors (Jones & Dangl, 2006). These effector molecules, sometimes called virulence factors, are usually associated with pathogenicity and are not essential to the pathogen, so they can evolve more quickly than pathogen-associated molecular patterns.

Heterozygous diploids are able to express two different recognition proteins at each locus, and so potentially double the PRR and R proteins available to recognize pathogens (Nuismer & Otto, 2004). This enhanced detection may also aid in the discrimination between parasites and beneficial symbionts, especially when the two are closely related, or even switch between roles (Wang et al., 2010). Diploidy may be particularly important for the success of the R proteins that recognize fast-evolving effector molecules, and indeed R genes are highly polymorphic (Dangl & Jones, 2001). Plants must engage in Red Queen dynamics with pathogens whose generation times are orders of magnitude shorter, so perhaps harboring alternate alleles of these genes allows them greater coverage of the potential phenospace of effectors. These immunity-related advantages of diploidy apply not only to plants but also to all organisms likely to be host to parasites, that is, long-lived, multicellular organisms that compose the main diploid clades of eukaryotes. The high rate of polymorphisms in host immune systems, whether adaptive (Messaoudi et al., 2002) or innate (Lazarus et al., 2002), shows the advantage to hosts of producing a wide array of recognition molecules.

2.3 Environments in which haploidy or diploidy is favored

The above discussion of characteristics and advantages of haploidy and diploidy already alluded to some

environmental conditions under which each condition is selected for. In general, haploidy seems to prevail in a homogeneous, nutrient/energy-limited environment. Aquatic habitats in the first one to two billion years of life's existence on earth seem to meet this description, as not enough solar energy had been fixed by photosynthesis (Kaufman & Xiao, 2003) and most mineral nutrients remained locked in the land crust (Schwartzman & Volk, 1989; Algeo et al., 2001). Prokaryotes and unicellular eukaryotes, presumably with haploidy-dominant life cycles, were indeed the main inhabitants of the earth during this period (Knoll, 2003).

Diploidy, given its innate ability to allow organisms to deal with variable environmental conditions and its potential to produce genetic diversity and evolutionary novelties in both short and long terms, is favored in a heterogeneous, nutrient-rich, and high energy environment. Colonization of the land by plants and other organisms during the early Phanerozoic Eon dramatically accelerated creation of this type of environment, as the rates of photosynthesis (Berner, 2001; Kaufman & Xiao, 2003) and rock weathering (Algeo et al., 2001) both increased significantly. The latter process not only affected the terrestrial ecosystem but also greatly enriched lakes and oceans through runoff and thereby changed environments for freshwater and marine organisms (Algeo et al., 2001), which might have spurred secondary and tertiary radiations of green, red, brown, and other algae and contributed to origins of the lineages that exhibit diploid generation-dominant life cycles (Fig. 1). The age of floridean red algae estimated from molecular data, around 580 million years, seems to be consistent with this idea (Yoon et al., 2004). Aquatic habitats before and after the origin of the land plant-initiated biosystem differ mainly in nutrient and energy levels. However, the terrestrial environment since the beginning of the Phanerozoic Eon almost certainly encountered another dimension of complexity: increased intensity of interaction among organisms. This is because the lower density of the physical medium, air, makes it much easier for organisms to interact with each other on land than in water. Recently, it has been suggested that life on earth entered into a period of "escalatory coevolution" since the Ediacaran as eumetazoans entered into the race, which fundamentally altered rules of the game in ecology through their invention of multitrophic food webs, large body size, life history trade-offs, ecological succession, biogeography, and major increases in standing biomass (Butterfield, 2007). Although this idea is appreciated, it is argued here that the triggering event was not the emergence of eumetazoans, but colonization of the land by one group of photosynthetic eukaryotes, namely plants, that changed the dynamics and rate of carbon cycling and energy flow on earth through their greatly increased photosynthetic capability. In addition, the highly mutagenic environment on land, caused by the high intensity of UV, selected for organisms that could deal with mutation pressure (through masking of deleterious alleles by dominance–recessiveness interaction). The three-phase physical environment on land (aboveground air, gas; soil, solid; and underground water, liquid), as opposed to the single-phase aquatic environment (except at the bottom), also created a higher dimensional and expanded phenospace, which would favor organisms that could generate more genotypic diversity. As discussed above, an extended diploid phase in the life cycle, which can afford a large number of meiocytes, seems to be the ontogenetic strategy to meet these environmental challenges. Finally, we wish to point out here that as more solar energy is retained on earth through photosynthesis, the environment becomes more heterogeneous entropically. The organisms that can generate more genetic diversity, both at the genome level by all kinds of mutations and during the expression of genetic information at the phenetic level through life cycle alterations and other developmental pathways, will be selected for in the new environment.

Although the above discussion of environmental conditions that favored haploidy and diploidy was from a macroevolutionary perspective, we wish to emphasize that the modern distribution of these two fundamental types of environments is heterogeneous both spatially and temporally, providing a fertile ground for microevolutionists to investigate the mechanisms and processes by which each genetic condition adapts to its respective environment. Even though the environment on earth undoubtedly evolves toward a direction of higher energy and more nutrients (unleashed from the terrestrial crust), the ancestral type of environment that was once more widespread when life was dominantly haploid has by no means disappeared. It not only persists but is also mixed with the derived type of environment that favors diploidy to create hybrid types, allowing evolution of organisms with diverse life cycle strategies as seen in modern eukaryotes (Fig. 1).

One case study of the life cycle in such hybrid environments on a microevolutionary scale has been provided recently by Frada et al. (2008). In the coccolithophore *Emiliana huxleyi*, a prymnesiophyte, different stages of the life cycle have allowed the organism to defend against viral attacks. The species is globally important in the carbon and calcium cycling, and undergoes large "blooms" in which the population spikes and then is decimated by viral epidemics. Originally, these viral epidemics were thought to follow Red Queen, kill-the-winner, dynamics. However, the same genotypes are

observed following these massive die-offs. Experimentation revealed that the species uses a life cycle-based defense strategy against the virus by retreating to a non-calcified haploid stage that the virus cannot recognize when it is present. This allows for selection of a diploid stage of the species to maximize its fitness in other evolutionary directions, such as metabolic optimization, rather than forcing the organism to enter classic Red Queen dynamics and become beholden to evolving defense against the virus at the expense of other fitness considerations (Frada et al., 2008).

3 Phylogeny

Inference of macroevolutionary patterns is dependent on phylogeny. In the earliest studies of life cycle evolution in land plants, a crude phylogeny of green algae and plants was used (Čelakovsky, 1874; Bower, 1890). Today we have much better knowledge of plant phylogeny, thanks to clarification of many conceptual issues in evolutionary biology and phylogenetic inference by cladistics (Hennig, 1966) and computational analyses of a massive amount of morphological and molecular data. Below we will briefly review current knowledge of the phylogeny of charophytes (a paraphyletic group that contains various green algal lineages, as discussed below) and land plants, with a focus on aspects relevant to understanding evolution of the life cycle.

Although green algae, specifically *Coleochaete*, were used to infer the ancestral condition of the land plant life cycle in the two prominent early studies (Čelakovsky, 1874; Bower, 1890) that formulated the antithetic hypothesis of origin of the sporophyte generation, it was not until the 1960s–70s that discovery of the phragmoplast, glycolate oxidase, and similar microtubular cytoskeletons of the spermatozoids or motile cells in some charophytic algae and land plants firmly established that these green algae represented the closest algal relatives of land plants (Pickett-Heaps, 1967, 1975; Stewart & Mattox, 1975). A specific clade of green plants, streptophytes, was identified (Jeffrey, 1967, 1982; Bremer & Wanntorp, 1981; Bremer, 1985), which now includes *Mesostigma* (Rogers et al., 1981; Melkonian, 1989; Lemieux et al., 2007), *Chlorokybus* (Rogers et al., 1980), Klebsormidiales (Floyd et al., 1972; Pickett-Heaps, 1972), Zygnematales (Fowke & Pickett-Heaps, 1969), Coleochaetales (Marchant & Pickett-Heaps, 1973), Charales (Pickett-Heaps, 1967), and embryophytes. More recent phylogenetic analyses of molecular and morphological data have confirmed this result (Manhart & Palmer, 1990; Graham et al., 1991; Karol et al., 2001; Turmel et al., 2007; Finet et al., 2010; Wodniok et al., 2011). Further, several analyses indicate that Charales are sister to land plants (Karol et al., 2001; Qiu et al., 2006, 2007), although there is still some controversy on this issue (Graham et al., 1991; Turmel et al., 2007; Finet et al., 2010; Wodniok et al., 2011).

The monophyly of land plants was only implicitly assumed in early studies of life cycle evolution by the antithetic school (Čelakovsky, 1874; Bower, 1890, 1908, 1935; Campbell, 1903, 1924; Smith, 1955), as evolutionary thinking in those days was not anywhere nearly as rigorous as today. However, Campbell (1903), in refuting the homologous hypothesis of alternation of generations, did list a number of features in archegonium structure/development and spore production that suggested a common origin of bryophytes, pteridophytes, and seed plants. The first explicit test of land plant monophyly came in the wave of cladistic analysis of morphological characters (Parenti, 1980; Mishler & Churchill, 1984; Kenrick & Crane, 1997), and the same result has been obtained by recent molecular phylogenetic analyses (Qiu et al., 2006, 2007; Finet et al., 2010). One particular question on relationships among basal land plants is critical for resolving the debate between antithetic and homologous hypotheses: whether bryophytes are a monophyletic or paraphyletic group. Both morphological (Parenti, 1980; Mishler & Churchill, 1984; Kenrick & Crane, 1997) and molecular (Qiu et al., 1998, 2006) studies have shown that paraphyly is more likely to be the correct hypothesis, even though a few studies have recovered a monophyletic group of bryophytes that is sister to vascular plants (Nishiyama et al., 2004; Goremykin & Hellwig, 2005; Finet et al., 2010; Wodniok et al., 2011). This latter result is likely an analytical artifact of unbalanced sampling of characters versus taxa, a common problem in phylogenomic analyses (Heath et al., 2008). Recently, another piece of phylogenetic evidence critical to solving the puzzle of alternation of generations in early land plants was recovered in several comparative structural studies (Frey et al., 2001; Carafa et al., 2005) and molecular phylogenetic analyses (Samigullin et al., 2002; Kelch et al., 2004; Groth-Malonek et al., 2005; Wolf et al., 2005; Qiu et al., 2006, 2007): hornworts were shown to be sister to vascular plants. This relationship was suggested more than a century ago (Campbell, 1895, 1903, 1924) and an elaborate evolutionary developmental scheme was even proposed for how a matrotrophic sporophyte in bryophytes made the transition to a free-living one in vascular plants (Smith, 1955), but these ideas were largely forgotten in recent literature, even though one of the first morphological cladistic studies recovered the topology (Parenti, 1980).

How relationships among basal lineages of vascular plants are resolved directly impacts interpretation of patterns and evolution of life cycle after the sporophyte changed from matrotrophic to free-living. One particular issue concerns the positions of Psilotaceae and lycophytes, that is, which group represents the sister group to all other extant vascular plants. Because Psilotaceae lack roots and have sporangia located at the ends of shortened axes, these superficial similarities to extinct early vascular plants (psilophytes and rhyniophytes) led some to believe that this group represents the oldest living vascular plant lineage (Parenti, 1980; Bremer, 1985) (also see Gifford & Foster, 1989). This topology would have profound implications on how the sporophyte gained the free-living status, how the sporangium and the structure bearing it evolved, and how the leaf evolved. One morphological study concluded that Psilotaceae were a member of the true ferns and not relicts of the oldest vascular plants (Bierhorst, 1968). Later, discovery of a rare rearrangement in the chloroplast genome shared by all extant vascular plants except lycophytes showed that it was lycophytes, not Psilotaceae, that occupied the key position of the sister group to all other extant vascular plants (Raubeson & Jansen, 1992). Further molecular phylogenetic analyses uncovered more evidence that supported placement of Psilotaceae, along with *Equisetum*, with ferns (Pryer et al., 2001; Qiu et al., 2006), with these groups forming a clade suggested earlier by a morphological cladistic analysis, monilophytes (Kenrick & Crane, 1997). Finally, this clade of monilophytes was shown to be sister to seed plants (Kenrick & Crane, 1997; Pryer et al., 2001; Qiu et al., 2006).

4 Evolution of the life cycle in land plants

It has been suggested that the life cycle is the central unit in biology and thus much of evolution can be viewed as the alteration of life cycles through time (Bonner, 1965). When the diverse life cycles of charophytic algae (van den Hoek et al., 1995) and land plants (Smith, 1955; Gifford & Foster, 1989; Crum, 2001; Raven et al., 2005) are overlaid on the current phylogeny, it becomes clear that the origin and evolution of land plants were essentially a process of expansion of the diploid phase from a single cell (as in Charales and other charophytic algae, but see a recent discussion on this topic elsewhere (Haig, 2010)) to a large free-living organism (as in vascular plants), accompanied by reduction of the haploid phase from bryophytes, to pteridophytes, and to seed plants (Fig. 2). At an early stage in land colonization by plants, the haploid phase did experience some degree of elaboration, to the point that several extinct stem relatives of vascular plants possessed well developed and complex diploid and haploid phases (Remy et al., 1993; Kenrick, 1994, 2000; Taylor et al., 2005; Gerrienne & Gonez, 2011), perhaps because the environment was more favorable for plants with a haploid phase-dominant life cycle. Below we will discuss several aspects of life cycle evolution that underlie major transitions in land plant evolution.

4.1 Origin of the diploid generation

One of the most important events in the evolution of photosynthetic life and life in general is the origin of land plants. It is probably no coincidence that such an event involved alteration of one of the most fundamental processes in biology, cell division, which through mutations led to a transition from uni- to multicellularity. Further, this seemingly simple transition was built upon another major innovation of eukaryotes, meiotic sex (meiosis and syngamy) (Maynard Smith & Szathmary, 1995), as in this case it happened at the diploid level. In streptophytes the uni- to multicellularity transition had happened once at the haploid level during an earlier stage, soon after emergence of the group (Qiu, 2008). An evolutionary explanation for such a transition was offered by Svedelius nearly a century ago (Svedelius, 1921, 1927), to exploit the potential of meiosis for generating genetic diversity. Organisms having more than one meiocyte per zygote (e.g., land plants) can outcompete those that have only one meiocyte, which is identical to the zygote (e.g., Charales), in a heterogeneous environment, because more genetically diverse offspring can be produced. By contrast, Bower's explanation that more spores are produced by a large sporophyte to ensure enough fertilization events in order for organisms to escape competition in water and survive/flourish on land (Bower, 1890, 1908, 1935) is of secondary importance, despite the fact that he was the first to explicitly suggest that the origin of the diploid generation in land plants was due to a delay of meiosis (Bower, 1908). It should be added here that Čelakovsky's (1874) thinking on this question was insightful even though a complete understanding of sexual reproduction had not been achieved at the time—meiosis had not yet been discovered (Van Beneden, 1883) and only fertilization was known. In formulating his antithetic hypothesis, Čelakovsky (1874) rightly recognized that sexual reproduction, as an advantageous means of regeneration of the species, compensated the defective effects accumulated during an individual's struggle for existence by bringing in relevant similar material of another parent. It was from this perspective that he astutely recognized the antithetic nature of the evolutionarily newly arisen sporophyte generation relative to the pre-existing

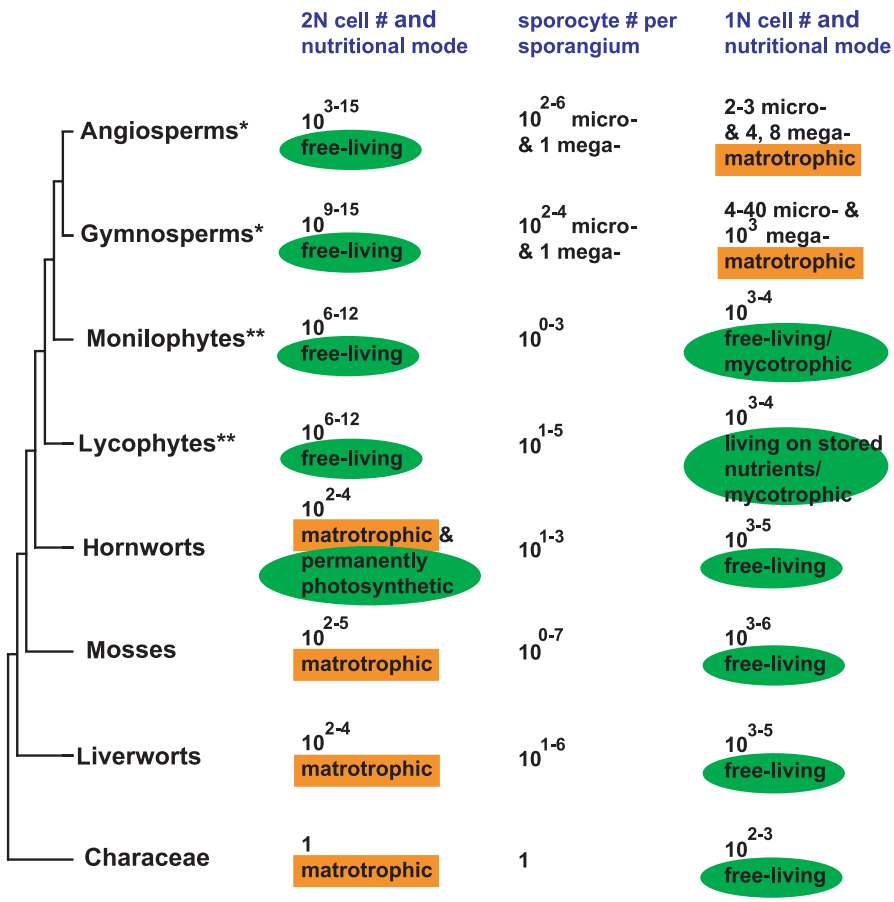

Fig. 2. Evolutionary trends of size and nutritional mode of the diploid and haploid phases and sporocyte (meiocyte) number per sporangium in land plants. The phylogeny is based on information reviewed in Qiu (2008). The information on cell numbers of sporophytes and gametophytes and sporocyte number was obtained as follows: bryophytes (Kreulen, 1972; Longton & Schuster, 1983); pteridophytes (Bower, 1897, 1900; Smith, 1900; Parkinson, 1987; Gifford & Foster, 1989); gymnosperms (Smith, 1907; Chamberlain, 1909; Singh, 1978; Gifford & Foster, 1989); and angiosperms (Walter, 1983; Gifford & Foster, 1989; Johri et al., 1992). The cell numbers in the large sporophytes of vascular plants represent a gross estimation. In vascular plants, the number of sporangia is more than one per sporophyte and fluctuates significantly as a function of the sporophyte size and physiological condition. For perennial species, the duration of reproductive length (more than 1 year) needs to be considered. *In angiosperm and gymnosperm gametophytes, the number of nuclei is counted, as the gametophytes are not always cellularized. **The sporocyte number per sporangium reflects both hetero- and homosporous conditions in lycophytes (*Isoetes* has ~50 meiocytes per megasporangium (Smith, 1900)) and monilophytes.

gametophyte generation. We now know that sexual reproduction involves meiosis and fertilization, and through both processes parental traits are mixed through recombination, independent assortment, and random union of gametes (Kondrashov, 1988; Maynard Smith & Szathmary, 1995).

The general significance of this post-zygotic increase in diploid cell number during colonization of the land by plants can perhaps be better appreciated when a broader perspective is taken. In fungal evolution, which shows some parallelisms to plant evolution in features such as immobility, spore production, and alternation of generations, colonization of the land was soon followed by a uni- to multicellularity transition at the diploid (actually dikaryotic) level when their life cycle diversity (Alexopoulos et al., 1996) is overlaid on the recently published molecular phylogeny (James et al., 2006). As plants, fungi, and animals constitute the three large multicellular eukaryote lineages that are mainly responsible for establishing the terrestrial ecosystem and animals were already diploid before coming onto land, it is perhaps safe to say that the trend shown by two of the three lineages during terrestrialization is a result of strong selective pressure rather than coincidence.

4.2 Diploid structure in bryophytes: What is it?

Once plants "set foot" on land and the diploid generation appeared, the challenge became to make the matrotrophic "sporophyte" free-living, as the haploid generation seems to be less fit to the heterogeneous land environment than the diploid one, as discussed above. In bryophytes, there has been some confusion about

what the diploid structure really corresponds to. In most modern literature, from cladistic analyses of morphological characters (Mishler & Churchill, 1984, 1985; Kenrick & Crane, 1997) to commonly used textbooks (Raven et al., 2005), it has been regarded as a sporophyte, an unbranched sporophytic plant with a single sporangium (Mishler & Churchill, 1984). Recently, this interpretation has been challenged. It has been argued that the diploid structure in bryophytes merely represents a sporogonium, that is, a footed sporangium, with the seta being a sporangium stalk, and that the shoot of a vascular plant, or more precisely a polysporangiophyte (sensu Bower, 1908; Kenrick & Crane, 1997), is a novel vegetative organ interpolated into the life cycle (Kato & Akiyama, 2005). In genetic mutants of moss *Physcomitrella patens* that had two sporangia on a sporophyte, no organ other than the sporangium and seta developed (Tanahashi et al., 2005). In older literature, a sporogonium is in fact what this structure was called (Čelakovsky, 1874; Bower, 1890, 1908, 1935; Campbell, 1895; Svedelius, 1927). The placement of hornworts, not mosses, as the sister group to vascular plants by recent comparative structural studies (Frey et al., 2001; Carafa et al., 2005) and molecular phylogenetic analyses (Samigullin et al., 2002; Kelch et al., 2004; Groth-Malonek et al., 2005; Wolf et al., 2005; Qiu et al., 2006) now casts doubt on a homology between conducting tissues in the seta of a moss and vascular tissues in the stem of a polysporangiophyte suggested by morphological cladistic studies (Mishler & Churchill, 1984), lending support to the sporogonium interpretation.

It may also be helpful to look at meristems involved in producing the diploid structures in bryophytes and vascular plants. In vascular plants, shoot and root apical meristems are essentially required for development of a vertical axis that is perpendicular to the ground surface and also bears lateral photosynthetic organs (leaves) and reproductive structures (sporangia) (Cooke et al., 2004), although some early vascular plants lacked leaves and their sporangia were terminal. The latter two types of organs are produced by the shoot apical meristem. The shoot and root apical meristems basically consist of a group of pluripotent cells that show either negative or positive gravitropic response (Qiu, 2008). In bryophytes, however, the root apical meristem is clearly lacking, as the diploid phase is matrotrophic on the gametophyte (Graham & Wilcox, 2000b). A "shoot apical meristem" is present, but is it equivalent or homologous to the shoot apical meristem in vascular plants? It produces only a seta and a sporangium, except in a few groups such as *Sphagnum* and *Archidium*, where the seta is lacking (Crum, 2001). Further, its negative gravitropic response is far from being universally established as in vascular plants. In many complex thalloid liverworts and *Blasia*, the sporogonium grows downward or horizontally, instead of upward as in other bryophytes (Schuster, 1992a, 1992b; Crum, 2001). Distribution of "shoot apical meristems" with these non-negative gravitropic responses in liverworts on the recently reconstructed phylogeny (Forrest et al., 2006; Qiu et al., 2006; 2007) suggests that either the negative gravitropic response was lost once in the common ancestor of *Blasia* and the complex thalloid liverworts and regained several times later, or lost many times independently. Regardless of the situation in this group of liverworts, the sporogonia in all other liverworts, mosses, and hornworts do grow upward, even in some leafy liverworts that produce an underground marsupium enclosing the sporogonium (Schuster, 1966; Crum, 2001) and in *Sphagnum* and *Archidium,* where the sporogonium lacks a seta and shows minimal upward growth (Crum, 2001). Thus, it is safe to say that while the "shoot apical meristem" in bryophytes is on an evolutionary trajectory to become a fully mature structure, it has not quite reached the ontogenetic stage found in vascular plants (Graham et al., 2000). Hence, it may be best not to equate the diploid structure of bryophytes to the entire sporophyte of vascular plants. This structure in bryophytes, especially in liverworts and mosses, is merely a spore-producing and dispersal organ, unlike the sporophyte of a vascular plant, which carries out full-scale functions of a free-living plant, including water and nutrient absorption and conduction, photosynthesis, and reproduction.

4.3 Hornworts and the transition from gametophyte to sporophyte as the dominant free-living generation

The diploid structure in hornworts is different from its counterpart in liverworts and mosses in several aspects. First, in terms of its size relative to the gametophyte, it is the largest among bryophytes, and the ratio of sporophyte : gametophyte size reaches nearly 1:1 (Schuster, 1992b). Second, this structure is permanently photosynthetic (Schuster, 1992b), and in fact there has been a report of photosynthate transfer from the sporophyte to the gametophyte (Stewart & Rodgers, 1977). Some mosses have partially photosynthetic sporophytes (Bold, 1940; Stark, 2002), but not to this extent. Third, hornworts have the longest-lived sporophyte among bryophytes, more than 9 months in one particular case (*Anthoceros fusiformis*), and it was almost free-living after the gametophytic tissues had collapsed (Campbell, 1924). Fourth, hornworts and pteridophytes both have stalkless archegonia sunken in gametophytes, whereas liverworts and mosses have stalked archegonia

positioned above the surface of gametophytes (Campbell, 1895, 1903; Smith, 1955; Doyle, 2012). Amazingly, the type of sunken archegonia found in extant hornworts and basal vascular plants have been observed in Devonian polysporangiophyte fossils from the Rhynie Chert (Remy et al., 1993; Kenrick & Crane, 1997; Taylor et al., 2005). Finally, hornworts and basal pteridophytes such as lycophytes and eusporangiate ferns have similar spore morphology (trilete spores) (Doyle, 2012) and possibly comparable conducting tissues in the sporophytes (Campbell, 1924; Proskauer, 1960) (but see a discussion on the topic elsewhere (Hébant, 1977)). Some of these similarities may not be synapomorphic for hornworts and vascular plants alone. For example, trilete spores are found in basal moss lineages such as *Takakia* (Renzaglia et al., 1997; Jia et al., 2003), *Sphagnum* (Brown et al., 1982), and *Andreaea* (Brown & Lemmon, 1984), in addition to hornworts and vascular plants (Gray, 1985; Kramer & Green, 1990; Tryon & Lugardon, 1991; Schuster, 1992b; Taylor, 2003; Steemans et al., 2009). A systematic and detailed survey is perhaps needed using the newly available phylogeny to re-evaluate the phylogenetic informativeness and the level of homoplasy in these characters.

In light of the recent results of several comparative structural studies (Frey et al., 2001; Carafa et al., 2005) and molecular phylogenetic analyses (Samigullin et al., 2002; Kelch et al., 2004; Groth-Malonek et al., 2005; Wolf et al., 2005; Qiu et al., 2006), which suggested that hornworts are the sister group of vascular plants, the aforementioned characters highlight hornworts as a prime transitional group that bridges the gap between other bryophytes and vascular plants. What is particularly striking is that several of these characters are involved in making a nutritionally independent sporophyte. Further, the sunken and stalkless archegonia in the completely thalloid gametophytes across the entire hornwort clade, in contrast to their stalked counterparts in liverworts and mosses, indicate gametophyte reduction and internalization of fertilization. Hence, the trends of life cycle evolution in land plants—sporophyte elaboration and gametophyte reduction—clearly began in hornworts, if not earlier, not in early vascular plants as usually believed.

4.4 Isomorphic versus heteromorphic alternation of generations and origin of the diplobiontic life cycle in basal vascular plants

During the bryophyte–vascular plant transition, one major change was the establishment of a free-living sporophyte. As this sporophyte gained nutritional and physiological independence, especially in producing a root system, the gametophyte initially remained as a free-living organism. The extant bryophytes and basal vascular plants show various life cycle conditions that document this transition. However, one major question has remained unanswered, namely, whether the land plants that first established a free-living sporophyte showed an isomorphic or heteromorphic alternation of generations. The answer to this question may help us to understand the environment in which vascular plants originated, and perhaps the way the sporophyte gained independence.

Gametophytes of all extant bryophytes, lycophytes, and monilophytes show two fundamentally different types of morphology: a thallus with little tissue differentiation and organogenesis, or an axial structure with "stem" and "leaf", and usually "root" development. It is based on this morphological criterion that life cycles have been classified as having isomorphic or heteromorphic alternation of generations. Very recently, a second criterion has been suggested, size of the plant (Gerrienne & Gonez, 2011). As discussed earlier in this report, size of the organism is constrained by the ploidy level (Cavalier-Smith, 1978) and affects the adaptability of the organism to different environmental conditions in terms of nutrient (Lewis, 1985) and moisture (McManus & Qiu, 2010) availability. Indeed, the largest gametophytes of land plants, those of *Dawsonia* and *Fontinalis* (both mosses and the latter an aquatic plant), are only about 65 and 100 cm tall (long), respectively. Hence, we think that size is an important factor to consider when comparing phenotypes of the two generations, in addition to morphology. Further, the size argument is supported by the fact that in organisms that show truly isomorphic alternation of generations, such as *Ulva* (green algae) and *Polysiphonia* (red algae), the gametophyte and sporophyte are similar in both size and morphology, and both generations live in the same aquatic environment (Bold & Wynne, 1985). Using these two criteria, some fossil taxa that were claimed to have an isomorphic alternation of generations, e.g., *Lyonophyton–Aglaophyton*, *Kidstonophyton–Nothia*, and *Langiophyton–Horneophyton* (Remy et al., 1993; Taylor et al., 2005), are now seen to exhibit a heteromorphic alternation of generations, because the size of the two generations is an order of magnitude different in spite of the gametophyte being axial (Gerrienne & Gonez, 2011). Moreover, extant pteridophytes such as Lycopodiaceae, Psilotaceae, Ophioglossaceae, and Stromatopteridaceae that have an axial gametophyte (Kenrick & Crane, 1997) should also be considered heteromorphic in their alternation of generations in terms of both morphology and size. From a broad comparative morphological perspective

of charophytic algae, bryophytes and vascular plants, it is perplexing that the axial body plan of plants has figured so prominently in the study of alternation of generations, because this type of body plan is present in gametophytes of Charales, *Haplomitrium* (a member of the basalmost liverwort lineage (Qiu et al., 2006; Qiu et al., 2007)), leafy liverworts, and mosses. It is likely that this body plan was established in the common ancestor of Charales and land plants once the gravitropic response had evolved (Qiu, 2008), even though it is best developed in seed plants (Cooke et al., 2004), as it allows plants to explore maximally the 3-D space on land that is filled with carbon dioxide and light.

From the above discussion, it is safe to say that land plants lack any clearly documented example, extant or extinct, of plants with a truly isomorphic alternation of generations, although the two generations were more similar anatomically in some fossil polysporangiophytes than they are in any living groups, with *Sciadophyton* awaiting further investigation (Kenrick, 1994, 2000). Occurrence of only a heteromorphic alternation of generations in all land plants is consistent with the ideas that the different generations occupy different niches, allowing plants to adapt to the terrestrial environment in a stepwise fashion (Stebbins & Hill, 1980; Keddy, 1981), and that bryophytes and pteridophytes represent transitional groups that are amphibious and have not fully adapted to the terrestrial environment as seed plants have (Bower, 1890).

4.5 Gametophyte reduction and evolution of the highly developed sexual reproductive system in seed plants

In the nearly century-long debate over the antithetic and homologous hypotheses, the focus was placed mostly on the origin and evolution of the sporophyte, with less attention paid to the evolution of the gametophyte. Although elaboration of the sporophyte is a fascinating evolutionary developmental story and many innovations are recorded in fossils and extant plants (Doyle, 2012), reduction of the gametophyte is equally interesting, providing materials for understanding how genetics, ontogeny, and environment interplayed to shape diversity of the life cycle in land plants.

When plants first colonized land, the terrestrial environment was almost certainly a barren and harsh one, with low levels of nutrients, poor water maintenance capacity, high soil surface temperature, and little biotic activity, and hence was uninhabitable for large multicellular organisms (Schwartzman & Volk, 1989; Mora et al., 1991; Algeo et al., 2001). As plants grew and diversified, environmental conditions changed in terms of soil nutrient levels and humus content (Schwartzman & Volk, 1989; Mora et al., 1991; Algeo et al., 2001), atmospheric CO_2 and O_2 concentrations (Berner, 2001), and surface temperature (Schwartzman & Volk, 1989; Beerling et al., 2001), all of which could affect the size, height, morphology, and physiology of plants. From the discussion earlier in this report on environments in which different ploidy conditions are selected (Cavalier-Smith, 1978; Lewis, 1985), it should not be surprising to see that the haploid gametophyte generation is dominant in the life cycle of bryophytes, which represent the first phase of land plant evolution (Gray, 1985, 1993; Edwards et al., 1995; Taylor, 1995; Strother et al., 1996; Wellman et al., 2003). Further, moderation of the terrestrial environment by plants and other large multicellular eukaryotes, especially animals and fungi, gradually selected plants that had an increasingly larger proportion of the sporophyte generation in their life cycle. Expansion of the sporophyte generation naturally means that the relative share of the gametophyte generation in the life cycle decreases, except in cases where the gametophyte has acquired a new function and hence its size increases, for example, the megagametophyte in gymnosperms. Among the three extant lineages of bryophytes, hornworts are now believed to be sister to vascular plants. The stalkless archegonia sunken in thalloid gametophytes of hornworts and pteridophytes (Campbell, 1895, 1903; Smith, 1955; Gifford & Foster, 1989; Doyle, 2012) indicate that gametophyte reduction began at the bryophytic level (Smith, 1955), in the common ancestor of hornworts and vascular plants, and that sex organs were prime targets of selection, as the gametophytes remained photosynthetic in most pteridophytes.

It is well known that a major reduction of gametophyte, relative to the size of sporophyte, occurred in the common ancestor of vascular plants as the sporophyte became free-living (Gerrienne & Gonez, 2011). However, the most dramatic reduction, in terms of both relative and absolute size, happened when heterospory evolved in the line leading to seed plants, with the exception of megagametophyte, the absolute size of which increased relative to the size of gametophyte in homosporous pteridophytes (see below). Heterospory has been deemed to be "the most iterative key innovation in the evolutionary history of the plant kingdom," as it evolved independently at least 11 times during vascular plant evolution (the condition of spores with different sizes in the same sporangium in bryophytes, anisospory, is not included here) (Bateman & Dimichele, 1994). However, this trait can be dissected into a series of more readily defined evolutionary innovations: bimodality of spore size; dioicy;

heterosporangy; endospory; monomegaspory; endomegasporangy; integumentation; lagenostomy; *in situ* pollination; *in situ* fertilization; pollen tube formation; and siphonogamy. Seed plants have the most complex kind of heterospory, with the last five innovations confined to them (Bateman & Dimichele, 1994; Kenrick & Crane, 1997). It is important to note that several of these innovations are conditioned upon gametophyte reduction, at least for the male gametophyte: bimodality of spore size; endospory; endomegasporangy; *in situ* pollination; and *in situ* fertilization (essentially internal fertilization), as otherwise large gametophytes would make these innovations physically difficult if not impossible.

There is one major exception in the trajectory of gametophyte evolution/reduction in vascular plants: the size of the gametophyte did not reduce, or even increased, in the megagametophyte of non-angiosperm seed plants. This exception is perhaps caused by the newly acquired function of the megagametophyte: nourishing the embryo, which, unlike in pteridophytes where the embryo becomes free-living shortly after full development, stays in the seed for quite some time. Hence, in these plants, the megagametophyte was selected for larger size while the microgametophyte reduction continued. Once the embryo-nourishing function was taken over by the newly evolved triploid endosperm in angiosperms, the megagametophyte reduction took place. The reason why the megagametophyte or triploid endosperm was selected for nourishing the embryo is perhaps related to the genetic compatibility between the embryo and the nourishing tissue. The sporophytic tissue from the previous generation is likely less compatible with the new sporophyte (Haig & Westoby, 1989). Recent reports of epigenetic reprogramming during gametophyte development in angiosperms (Slotkin et al., 2009; Feng et al., 2010; Olmedo-Monfil et al., 2010) seem to support this view.

As can be seen from the diversity, biomass, and ecological service of modern seed plants, the evolutionary implications of heterospory are enormous, and indeed much has been written about them (see Bateman & Dimichele, 1994 and references therein). Here we will discuss only one set of aspects that have been relatively neglected in literature: meiosis, fertilization, and bimodality of spore and gamete number.

In the eukaryotic sexual reproductive system, two fundamental processes are meiosis and fertilization/syngamy. Meiosis is the step where mutations can arise through DNA replication errors, and much of the potential genetic diversity within a species is realized through recombination and independent assortment. Fertilization can also realize some of the genetic diversity within a species through random or non-random (selective outcrossing) union of gametes. It is also a critical step that determines the quality of the zygote and its immediate growth. In protists and many basal lineages of plants, animals, and fungi, the division of labor for reproduction is less pronounced. Hence the sex expression system is not very well developed. Bryophytes are almost always homosporous (in size and morphology), even when spores of two sexes, determined by sex chromosomes, are produced within a single sporangium (Smith, 1955; Tanurdzic & Banks, 2004). Most pteridophytes are homosporous with hormone-regulated sex differentiation (Tanurdzic & Banks, 2004). Some pteridophytes are heterosporous, but they do not show all the aspects of heterospory that are seen in seed plants.

Heterospory in seed plants represents one of the most developed sexual reproductive systems in eukaryotes, which is reflected by the degree of specialization of the male and female sexes in carrying out their respective roles in realizing the benefits of meiosis and fertilization. In the microsporangium, there are a large number of meiocytes, and the long- and short-term benefits of meiosis are realized in the form of mutations through DNA replication errors and recombination/independent assortment. In the megasporangium, only a single meiocyte undergoes meiosis, producing a single megagametophyte with three other megaspores aborted. This extreme reduction in megaspore number, not megagametophyte size, on the female side allows nourishment of one to several eggs (one in each archegonium), whose size is large and quality presumably high. In angiosperms, the reduction of female gametophyte is even more extreme, with only four to eight nuclei (Gifford & Foster, 1989; Friedman & Williams, 2003) (Fig. 2). Given that double fertilization in angiosperms involves two or three nuclei, one egg and one or two polar nuclei, there is not much room for further reduction in evolution. This situation approaches the haploid phase reduction on the female side in humans and other animals, with just one cell (egg), which is all that is needed for sexual reproduction. Also in angiosperms, enclosure of ovules inside an ovary allows evolution of stigmatic germination of pollen and pollen selection through stigma/style regulated self-incompatibility and exclusion of pollen from related diverged species (Doyle & Donoghue, 1986; Takayama & Isogai, 2005). In addition, double fertilization leads to production of a genetically compatible but meiotically handicapped (triploid) and hence evolutionarily disadvantageous endosperm to provide a specialized function of nourishing the zygote (Haig & Westoby, 1989; Friedman, 1995; Stewart-Cox et al., 2004). Both of these processes provide opportunities for enhancing the role of the female in realizing the

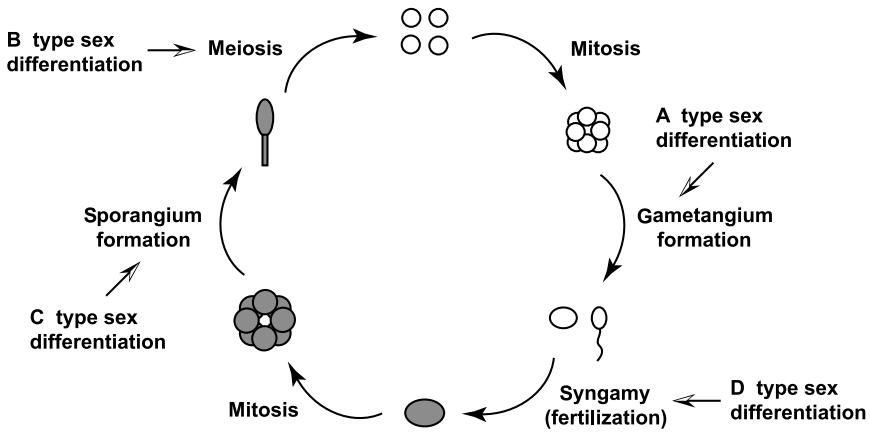

Fig. 3. Schematic diagram depicting the life cycle of sexually reproducing land plants. The filled gray cells are diploid and the open cells are haploid. Sex differentiation can occur during gametangium formation (in homothallic bryophytes and homosporous pteridophytes) (A type), meiosis (within a sporangium of heterothallic bryophytes with sex chromosomes) (B type), mega- and microsporogium formation (at the tissue/organ level among sporangia on an individual of seed plants without sex chromosomes and heterosporous pteridophytes) (C type), or fertilization (at the organismal level among individuals of dioecious seed plants with sex chromosomes) (D type sex determination).

benefits of fertilization, promoting outcrossing, setting up an arena for pollen/sperm competition, and ensuring the best care of the embryo. We wish to emphasize that the highly developed sexual reproduction system in seed plants and especially in angiosperms could not have evolved unless the gametophyte was reduced, because otherwise endospory could not evolve, there would not be sufficient resources to produce a large number of 1000s–10000s-celled gametophytes within a single sporangium, and *in situ* pollination and fertilization could not evolve. Finally, it is worth noting that in seed plants both micro- and megagametophytes are so reduced that they have ceased to carry out photosynthesis as do gametophytes in most pteridophytes, and that their main functions are to carry genetic information inside their cells and to store nutrients for the next generation of sporophytes in the case of megagametophytes.

The eukaryotic sexual reproductive system has some evolutionarily ancient components that are likely shared by almost all eukaryotes, such as meiosis and syngamy, and some young components that evolved independently in individual lineages, for example, sex chromosomes or other sex-determining mechanisms (Maynard Smith & Szathmary, 1995). Hence, it may help to understand how the heterospory-mediated sex expression system in plants evolved by comparing it with the similarly highly developed sex expression system in mammals, and looking at it from a broad perspective of life cycle and reproductive strategy evolution in terrestrial multicellular eukaryotes in general. In animals, sex chromosomes evolved many times independently, but in mammals only twice, once in monotremes and the other in the common ancestor of marsupial and placental mammals (Wilson & Makova, 2009). It is the X–Y sex chromosome-controlled sex expression system in marsupial and placental mammals that shows some similarities with the heterospory-mediated sex expression system in seed plants. In both cases, a mutational step has been inserted in the life cycle before meiocyte formation to developmentally alter the number of meiocytes (similar to C and D types of sex expression in Fig. 3). Consequently, in mammals, the male and the female have dramatically different meiocyte numbers, with the ratio of sperm/egg reaching 5×10^6 in humans (Campbell & Reece, 2002) (this ratio could be 1000 times higher as the 400 000 potential egg cells in the female infant, not the several hundred eggs released during a woman's reproductive years, were considered here in calculation). This asymmetry in meiocyte number between the male and the female is probably responsible for a phenomenon dubbed male-driven evolution in mammals, as the rates of mutations in these animals seem to be dictated by those on the male side (Miyata et al., 1987; Li et al., 2002). However, we wish to point out that, as in seed plants, the female in mammals plays a role in selection of the egg and nourishment of the zygote, both processes being responsible for selection of mutations to be fixed. Hence the male-driven evolution hypothesis is inaccurate and should be modified as the male-driven mutation and the female-dictated selection hypothesis.

Heterospory in seed plants actually represents a type of sexual dimorphism that is determined by sex chromosomes, hormones, or environmental cues. The sex difference is manifested at the individual diploid level only when there are sex chromosomes (D type sex differentiation in Fig. 3). In most seed plants, however, there are no sex chromosomes and sex expression is

regulated at the tissue/organ level (C type in Fig. 3). Only in organisms with these two types of sex determination is it possible to regulate meiocyte number to allow division of labor for reproduction to develop. In organisms with sex differentiation occurring during meiosis or gametangium formation (A and B types in Fig. 3), it is developmentally too late to change meiocyte number in the different sexes. It is no coincidence that these two latter types are found in early land plants, bryophytes and pteridophytes, and the former two types are predominant in seed plants. The similarity between the seed plant and mammal reproductive strategies in having a large number of male meiocytes but a very small number of female meiocytes developed this way independently by moving the sex determination steps closer to the fertilization event that conceived the diploid individual. For organisms that have sex determination during or after meiosis, it is impossible to allow this specialization. At present, not enough is known about sex determination in angiosperms (Tanurdzic & Banks, 2004) and virtually no attention has been paid to the origin of heterospory in seed plants in the plant evo-devo community. Research on MADS box genes may shed light on this question if sufficient work is carried out on extant gymnosperms and pteridophytes that show some kind of heterospory, such as *Selaginella, Isoetes,* and water ferns (Theissen et al., 2000).

The reduction of the gametophyte in land plants left the haploid generation constrained to a solely sexual function, which is well suited to the physiology and genetic dynamics of this generation. As a result, seed plants, and angiosperms in particular, were able to achieve very sophisticated heterosporous reproductive systems. As the gametophyte was being reduced to its smallest useful form in land plants, the sporophyte generation was optimized to a role that best fits the constraints and strengths inherent in diploid physiology, using its larger size and advantages in interspecific interactions, metabolism, and resisting mutagenicity to navigate the terrestrial environment and provide the nutrients and energy the gametophyte needed.

4.6 Sporophyte elaboration and evolution of sporangium-bearing structures

The sporophyte generation is ontogenetically defined by fertilization and meiosis (sporogenesis). It does not exist in Charales and other charophytic algae that are known to have sexual reproduction, because the zygote never divides mitotically before it goes through meiosis (Graham, 1993; van den Hoek et al., 1995) (Fig. 2). In land plants, this generation arises as a result of delay of meiosis (Bower, 1908), but with developmental programs of varying length producing diploid structures with different sizes and morphologies in different lineages. The overall trend of sporophyte evolution is the steady increase of size and tissue/organ complexity, even though there are many cases of secondary reduction in size and complexity (Fig. 2). Most past and present morphological and developmental studies have devoted much attention to root, leaf, seed, flower, and other organs of the sporophyte. In this review, we will focus on the sporangium and the sporangium-bearing structures. The sporangium as an organ evolved *de novo* at the beginning of land plant evolution, and in fact has been preserved in some of the earliest fossil records of land plants (Wellman et al., 2003). In liverworts, mosses, and hornworts, the sporophyte generation is manifested in the form of a sporogonium, a spore-producing and -dispersing organ of the plant. Across land plants, the sporangium is the only organ of the sporophyte that is common to all lineages, and it is defined by a mass of sporogenous tissue and a protective layer (Bower, 1908). It can be regarded as another major synapomorphy of embryophytes (Parenti, 1980) after the embryo (Mishler & Churchill, 1984).

In all three bryophyte lineages, only one sporangium is produced in the sporophyte generation, and there are multiple sporangia on each gametophyte as a result of multiple independent fertilization events. Adaptation-wise, this morphological form is clearly preferred over the form of one gametophyte nutritionally supporting a single sporophyte with multiple sporangia, as several sperms, likely of different genetic makeups, are involved in fertilizing the genetically identical eggs (Renzaglia et al., 2000). It has been suggested that the sporophyte development in bryophytes is ontogenetically arrested at the sporogonium stage (Kato & Akiyama, 2005), and there seems to be some support from a developmental study of moss *Physcomitrella patens*, in which mutants with double sporangia on a sporophyte were generated and no organ beyond the sporangium and seta developed (Tanahashi et al., 2005), similar to the situation that has been found in nature (Bower, 1935). In hornworts the sporophytic developmental program may be slightly extended (Campbell, 1924). Because the polysporangiate state represents a clearly derived condition during the bryophyte–vascular plant transition, there has long been an interest in understanding how it arose (Bower, 1908; Kenrick & Crane, 1997). Among extant plants, there seems to be a large gap between the unisporangiate leafless sporophyte of bryophytes, which is nourished by the gametophyte, and the polysporangiate sporophyte of vascular plants, which is nutritionally supported by leaves (*Psilotum* and *Equisetum* represent a secondarily derived condition of

having photosynthetic stems and no or reduced leaves). Interestingly, this gap is filled by extinct Devonian taxa such as *Aglaophyton*, *Cooksonia, Horneophyton,* and *Rhynia,* which all have small (15–20 cm tall) and leafless polysporangiate sporophytes (Bower, 1935; Kenrick & Crane, 1997; Taylor et al., 2005; Gerrienne et al., 2006; Gerrienne & Gonez, 2011), nutritionally supported by the gametophyte (Boyce, 2008) or photosynthetic axes/sporangium stalks (Gensel, 2008). It may be speculated that these plants were once the fittest in an environment that was unfavorable for existence of leaves because of higher atmospheric CO_2 concentration, higher surface temperature (Beerling et al., 2001), and less water-holding soil, and that as environmental conditions changed (Schwartzman & Volk, 1989; Algeo et al., 2001; Berner, 2001), sporophytic leaves (with a large number of stomata) evolved and new taxa drove older leafless forms into extinction.

In extant vascular plants, sporangia are always leaf-borne, sometimes with stalks and other times sessile (Gifford & Foster, 1989). Further, the number of sporangia per sporophyte increases from one to many. As discussed above, there was a phase in the bryophyte–vascular plant transition that is represented by extinct stem-borne polysporangiate plants. Hence, how leaves evolved and sporangia changed from branch- to leaf-borne can probably only be studied by considering both extinct plants such as *Cooksonia* and *Rhynia* and extant lycophytes and basal euphyllophytes. Once leaf-borne sporangia evolved, some plants stayed at this level of sporangium-bearing architecture (e.g., some lycophytes and all monilophytes), and others evolved another level of more complex sporangium-bearing structures, strobili (e.g., some lycophytes, *Equisetum,* and seed plants).

The final step in evolution of sporangium-bearing structures took place during the origin of angiosperms, when sporophylls became more specialized in morphology, ovules were covered by sporophyll tissue, and sterile largely non-photosynthetic leaves (sepals and petals) evolved so that outcrossing could be maximized (Doyle & Donoghue, 1986; Takayama & Isogai, 2005). When considered jointly with the previous section, it can be appreciated that sporophyte elaboration proceeded along with gametophyte reduction, which together resulted in evolution of one of the most sophisticated reproductive systems in eukaryotes.

One aspect of sporophyte evolution that has received surprisingly little attention in recent literature is the number of sporocytes per sporangium. This number equals the number of meiotic events within each sporangium. When the number of sporangia produced by each zygote is considered, it can help shed light on two important evolutionary aspects of a species, the long-term evolutionary rate and the short-term ability of organisms to realize the potential genetic diversity of the species. In this review, we found that the sporocyte number per sporangium varies surprisingly little among major lineages of land plants (Fig. 2). As soon as the sporangium originated at the beginning of land plant evolution, it reached its maximal spore-producing capacity, as can be seen from the range of sporocyte number per sporangium in liverworts and mosses. In fact, the lower end of the range in liverworts and mosses, found in *Riccia gougetiana* and *Archidium alternifolium*, with only 192 and 16 sporocytes, respectively (Kreulen, 1972; Longton & Schuster, 1983), approaches the value reached by megasporocytes when heterospory evolved and megasporocyte number dramatically decreased, as in *Isoetes*, with only approximately 50 megasporocytes (Smith, 1900), and *Selaginella*, with one to several megasporocytes (Gifford & Foster, 1989; Schulz et al., 2010). Not surprisingly, the size of spores in *R. gougetiana* and *A. alternifolium* almost reaches that of *Isoetes* and *Selaginella* megaspores (Tryon & Lugardon, 1991). This might indicate that the genes controlling sporocyte number reduction during the origin of heterospory in vascular plants were already present in bryophytes. On the other hand, despite stagnation of the maximal sporocyte number per sporangium throughout land plant evolution, the number of sporocytes per zygote/sporophyte increased steadily because sporophyte size and longevity increased, with the exception of secondary reduction of these two features in herbaceous angiosperms (Fig. 2). This trend could suggest that evolutionary rate increased as land plants evolved, as replication errors that arose during premeiotic DNA synthesis were the main source of mutations, which has been deemed to be the fundamental driving force of evolution (Morgan, 1932; Nei, 2007). In addition, increase of both genome size (Leitch et al., 2005) and meiotic events per zygote/sporophyte allowed plants to produce more genetically diverse offspring as land plants evolved, providing more raw material in each generation for natural selection and contributing to fixation of better mutations. The argument that bryophytes have more sporangia produced with different sperm donors on each plant (Renzaglia et al., 2000) does not help much here because the small genome size imposes a significant limit on the diversity of spores that can be produced during meiosis. Therefore, increase of the sporocyte number per zygote/sporophyte during land plant evolution is an important mechanism that might have affected the long- and short-term evolutionary dynamics as these organisms adapted to the terrestrial environment.

5 Future prospects

After a hiatus of more than 50 years, the life cycle is coming back to the forefront of research in plant and evolutionary biology. In particular, progress made over the past several decades in understanding land plant phylogeny provides an opportunity to examine macroevolutionary trends of plant diversification. The questions asked by classical biologists about pattern and developmental mechanisms of the life cycle (Čelakovsky, 1874; Campbell, 1895; Bower, 1908, 1935; Smith, 1955; Bonner, 1965) can now be approached experimentally by comparative genomics and evolutionary developmental biology. Further, evolutionary ecology and paleobotany will provide the necessary knowledge on environmental changes to help understand how the drama of life cycle evolution unfolded over the past several hundred million years.

One area of research that is lacking is characterization of basic life history traits and strategies in different major clades of land plants. We attempted to examine the evolutionary trend in sporocyte number per sporangium and per zygote in this review (Fig. 2). The information was surprisingly scarce, and many studies were carried out over a century ago. As the overall phylogeny of land plants is relatively well known (Raubeson & Jansen, 1992; Bowe et al., 2000; Chaw et al., 2000; Goffinet et al., 2001; Pryer et al., 2001; He-Nygren et al., 2004; Heinrichs et al., 2005; Forrest et al., 2006; Qiu et al., 2006, 2010; Duff et al., 2007; Cox et al., 2010; Soltis et al., 2011), if a small to moderate number of species are investigated in each clade, ancestral and derived conditions in key life history characters, and the number of secondary reversals and independent origins can be relatively easily determined. Tracing the life cycle systematically across major lineages of land plants reveals the following list of characters or processes that are presumably homologous and deserve to be investigated.

Starting from fertilization, the egg:sperm ratio is likely to change going from water-dependent to water-independent fertilization, from homospory to heterospory, from wind to insect pollination, and from selfing to outcrossing. Second, embryo development may change when it is supported by a free-living gametophyte, a gametophyte matrotrophic on a sporophyte, or an angiosperm endosperm. Third, the timing of meiosis and the number of meiocytes per sporangium and per zygote clearly vary from lineage to lineage. The meiocyte number per zygote is an especially interesting character, as it may be partly responsible for determining evolutionary rate of a species. Thus far, the generation time of a species has been shown to be a main life history character that affects evolutionary rate (Laird et al., 1969; Li, 1997; Smith & Donoghue, 2008), yet the well-known conflicts between fossil and molecular dating analyses suggest that not all factors controlling the rate have been understood. Fourth, development of the gametophyte differs significantly between the free-living gametophyte of bryophytes and pteridophytes and the matrotrophic one in seed plants. This is one of the most fascinating aspects of plant development, and it cannot be studied in most other eukaryotic model organisms. This subject has received some attention recently from developmental biologists working on flowering plants (Slotkin et al., 2009; Feng et al., 2010; Olmedo-Monfil et al., 2010), but comparative information from non-flowering plants is urgently needed. Finally, gametogenesis differs in water-dependent external fertilization and pollen tube-mediated internal fertilization.

The second area that is likely to see breakthroughs in the next decade and to enhance our understanding of the development and evolution of the land plant life cycle is evolutionary developmental studies of various genes regulating the timing of meiosis and gametophyte/gamete development. One gene, *mei2,* has been shown in *Schizosaccharomyces pombe* to play an essential role in premeiotic DNA synthesis and the commitment to meiosis (Iino & Yamamoto, 1985; Watanabe & Yamamoto, 1994). Homologs of this gene exist in green algae and land plants (Jeffares et al., 2004), where they have experienced a few duplications events (Xue J-Y & Qiu Y-L, 2011, unpublished data). One subfamily of this gene has been shown to be involved in cell differentiation, expressed in shoot and root meristems rather than in cells undergoing meiosis (Veit et al., 1998; Jeffares et al., 2004). Another subfamily plays a role in the vegetative meristem and also in meiocytes of *Arabidopsis thaliana* (Kaur et al., 2006).

The development of the gametophyte and gametes, on the other hand, has most recently been shown to be controlled not just by regular genes, but also by epigenetic reprogramming. In angiosperms, for both male and female gamete development, associated cells or nuclei play an important role in supplying small RNAs for transposon silencing in the gametes, for which genome integrity without rampant transpositions of transposons seems to be essential during development and fertilization (Slotkin et al., 2009; Feng et al., 2010; Olmedo-Monfil et al., 2010). Because gametophytes in gymnosperms, pteridophytes, and bryophytes are generally larger and show higher levels of cell and tissue differentiation, how epigenetic reprogramming takes place during gametogenesis is currently unknown and will be a fertile area for exploration.

The third area to explore is synthetic evolutionary analyses of plant life cycle diversification patterns through combined studies in evolutionary ecology, developmental genetics, and phylogenetics. Two specific subfields of ecology could guide these analyses. One is ecosystem ecology, which explicitly divides interactions between organisms and their biotic and abiotic environments into nutrient cycling and energy flow (Lindeman, 1942; Odum, 1969, 1988; Odum & Barrett, 2005). Ecosystem analyses usually focus on metabolic aspects of organisms and their interactions with the environment in one time slice. If these analyses are extended from the present to the past, consideration of information flow, specifically genetic information, becomes necessary. Availability of reconstructed organismal phylogenies now renders this kind of analysis possible. The life cycle and its various parameters, such as time of meiosis initiation, meiocyte number, and egg:sperm ratio, deserve special attention in such comparative analyses.

The study of phylogenetic niche conservatism (Wiens, 2004; Losos, 2008) is another subfield that could facilitate growth of synthetic evolutionary studies of the life cycle. In pre-cladistic times, botanists often engaged in holistic thinking about plant evolution and interaction between plants and their environment (Čelakovsky, 1874; Campbell, 1903; Bower, 1908, 1935; Svedelius, 1927; Smith, 1955; Schuster, 1981). With some key evolutionary concepts clarified by cladistics, it is perhaps time to carry out evolutionary analysis by considering both clades and grades. Hence, it will be desirable to define evolutionary niches of various clades of charophytic algae and land plants and also to consider levels of phylogenetic niche conservatism within and across these clades. Specifically, clades within three grades need be considered: charophytic algae in a fundamentally aquatic environment; bryophytes and perhaps pteridophytes in a wet terrestrial and generally nutrient-poor environment; and seed plants in a dry and more nutrient-rich environment. The following aspects need to be considered when an evolutionary niche is defined: carbon-fixing rate (C3, C4, or CAM photosynthesis); nutrient-cycling rate (substrate type); ratio of photosynthetic area:total surface area of the plant (Beerling et al., 2001); rate of litter decay (Cornwell et al., 2008); genome size (gene repertoire) (Leitch et al., 2005); ploidy level of the free-living phase of the organism (Cavalier-Smith, 1978); meiocyte number; egg:sperm ratio; and mating strategy. The study of evolutionary niches for organisms and lineages is likely to bring organismal biology to the forefront of biology again after a century of biological research dominated by reductionistic approaches.

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References

Adl SM, Simpson AGB, Farmer MA, Anderson OR, Barta JR, Bowser SS, Brugerolle G, Fensome RA, Fredericq S, James TY, Karpov S, Kugrens P, Krug J, Lane CE, Lewis LA, Lodge J, Lynn DH, Mann DG, McCourt RM, Mendoza L, Moestrup O, Mozley-Standridge SE, Nerad TA, Shearer CA, Smirnov AV, Spiegel FW, Taylor MFJR. 2005. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. Journal of Eukaryotic Microbiology 52: 399–451.

Alexopoulos CJ, Mims CW, Blackwell M. 1996. Introductory mycology. New York: John Wiley & Sons.

Algeo TJ, Scheckler SE, Maynard JB. 2001. Effects of the middle to late Devonian spread of vascular land plants on weathering regimes, marine biotas, and global climate. In: Gensel PG, Edwards D eds. Plants invade the land: Evolutionary and environmental perspectives. New York: Columbia University Press. 213–236.

Andersen RA. 2004. Biology and systematics of heterokont and haptophyte algae. American Journal of Botany 91: 1508–1522.

Andersson JO, Sarchfield SW, Roger AJ. 2005. Gene transfers from Nanoarchaeota to an ancestor of diplomonads and parabasalids. Molecular Biology and Evolution 22: 85–90.

Baldauf SL. 2003. The deep roots of eukaryotes. Science 300: 1703–1706.

Baldauf SL. 2008. An overview of the phylogeny and diversity of eukaryotes. Journal of Systematics and Evolution 46: 263–273.

Baldauf SL, Bhattacharya D, Cockrill J, Hugenholtz P, Pawlowski J, Simpson AGB. 2004. The tree of life: An overview. In: Cracraft J, Donoghue MJ eds. Assembling the tree of life. New York: Oxford University Press. 43–75.

Bateman RM, Dimichele WA. 1994. Heterospory: The most iterative key innovation in the evolutionary history of the plant kingdom. Biological Reviews 69: 315–417.

Beerling DJ, Osborne CP, Chaloner WG. 2001. Evolution of leaf-form in land plants linked to atmospheric CO_2 decline in the Late Palaeozoic era. Nature 410: 352–354.

Bell G. 1994. The comparative biology of the alternation of generations. In: Kirkpatrick M ed. Lectures on mathematics in the life sciences: The evolution of haploid–diploid life cycles, 25. Providence: American Mathematical Society. 1–26.

Berner RA. 2001. The effect of the rise of land plants on atmospheric CO_2 during the Paleozoic. In: Gensel PG, Edwards

D eds. Plants invade the land: Evolutionary and environmental perspectives. New York: Columbia University Press. 173–178.
Bierhorst DW. 1968. On the Stromatopteridaceae (fam. nov.) and on the Psilotaceae. Phytomorphology 18: 232–268.
Bold HC. 1940. The nutrition of the sporophyte in the Musci. American Journal of Botany 27: 318–322.
Bold HC, Wynne MJ. 1985. Introduction to the algae. Engelwood Cliffs: Prentice-Hall.
Bonner JT. 1965. Size and cycle: An essay on the structure of biology. Princeton: Princeton University Press.
Bowe LM, Coat G, dePamphilis CW. 2000. Phylogeny of seed plants based on all three genomic compartments: Extant gymnosperms are monophyletic and Gnetales' closest relatives are conifers. Proceedings of the National Academy of Sciences USA 97: 4092–4097.
Bower FO. 1890. On antithetic as distinct from homologous alternation of generations in plants. Annals of Botany 4: 347–370.
Bower FO. 1897. Studies in the morphology of spore-producing members. III. Marattiaceae. Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences 189: 35–81.
Bower FO. 1900. Studies in the morphology of spore-producing members. IV. The leptosporangiate ferns. Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences 192: 29–138.
Bower FO. 1908. The origin of a land flora: A theory based upon the facts of alternation. London: MacMillan.
Bower FO. 1935. Primitive land plants, also known as the Archegoniatae. London: MacMillan.
Boyce CK. 2008. How green was *Cooksonia*? The importance of size in understanding the early evolution of physiology in the vascular plant lineage. Paleobiology 34: 179–194.
Bremer K. 1985. Summary of green plant phylogeny and classification. Cladistics 1: 369–385.
Bremer K, Wanntorp H-E. 1981. A cladistic classification of green plants. Nordic Journal of Botany 1: 1–3.
Brown RC, Lemmon BE. 1984. Spore wall development in *Andreaea* (Musci: Andreaeopsida). American Journal of Botany 71: 412–420.
Brown RC, Lemmon BE, Carothers ZB. 1982. Spore wall ultrastructure of *Sphagnum lescurii* Sull. Review of Palaeobotany and Palynology 38: 99–107.
Butterfield NJ. 2007. Macroevolution and macroecology through deep time. Palaeontology 50: 41–55.
Campbell DH. 1895. The structure and development of the mosses and ferns (Archegoniatae). London: MacMillan.
Campbell DH. 1903. Antithetic versus homologous alternation. American Naturalist 37: 153–169.
Campbell DH. 1924. A remarkable development of the sporophyte in *Anthoceros fusiformis*, Aust. Annals of Botany 37: 473–483.
Campbell NA, Reece JB. 2002. Biology. San Francisco: Benjamin Cummings.
Carafa A, Duckett JG, Knox JP, Ligrone R. 2005. Distribution of cell-wall xylans in bryophytes and tracheophytes: New insights into basal interrelationships of land plants. New Phytologist 168: 231–240.
Cavalier-Smith T. 1978. Nuclear volume control by nucleoskeletal DNA, selection for cell volume and cell growth rate, and the solution of the DNA C-value paradox. Journal of Cell Science 34: 247–278.
Čelakovsky L. 1874. Ueber die verschiedenen Formen und die Bedeutung des Generationwechsels der Pflanzen. Sitzungsberichte der koeniglichen Boehmischen Gesellschaft der Wissenschaften in Prag 2: 21–61.
Chamberlain CJ. 1909. Spermatogenesis in *Dioon edule*. Botanical Gazette 47: 215–236.
Chaw SM, Parkinson CL, Cheng YC, Vincent TM, Palmer JD. 2000. Seed plant phylogeny inferred from all three plant genomes: Monophyly of extant gymnosperms and origin of Gnetales from conifers. Proceedings of the National Academy of Sciences USA 97: 4086–4091.
Coelho SM, Peters AF, Charrier B, Roze D, Destombe C, Valero M, Cock JM. 2007. Complex life cycles of multicellular eukaryotes: New approaches based on the use of model organisms. Gene 406: 152–170.
Cooke TJ, Poli D, Cohen JD. 2004. Did auxin play a crucial role in the evolution of novel body plans during the Late Silurian–Early Devonian radiation of land plants? In: Hemsley AR, Poole I eds. The evolution of plant physiology: From whole plants to ecosystems. Amsterdam: Elsevier Academic Press. 85–107.
Cornwell WK, Cornelissen JHC, Amatangelo K, Dorrepaal E, Eviner VT, Godoy O, Hobbie SE, Hoorens B, Kurokawa H, Perez-Harguindeguy N, Quested HM, Santiago LS, Wardle DA, Wright IJ, Aerts R, Allison SD, van Bodegom P, Brovkin V, Chatain A, Callaghan TV, Diaz S, Garnier E, Gurvich DE, Kazakou E, Klein JA, Read J, Reich PB, Soudzilovskaia NA, Vaieretti MV, Westoby M. 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecology Letters 11: 1065–1071.
Cox JC, Goffinet B, Wickett NJ, Boles SB, Shaw AJ. 2010. Moss diversity: A molecular phylogenetic analysis of genera. Phytotaxa 9: 175–195.
Crow JF, Kimura M. 1965. Evolution in sexual and asexual populations. American Naturalist 99: 439–450.
Crum H. 2001. Structural diversity of bryophytes. Ann Arbor: The University of Michigan Herbarium.
Crum HA, Anderson LE. 1981. Mosses of eastern North America. New York: Columbia University Press.
Dangl JL, Jones JD. 2001. Plant pathogens and integrated defence responses to infection. Nature 411: 826–833.
Doyle JA. 2012. Phylogenetics and morphological innovations in land plants. In: Ambrose B, Purugganan MD eds. The evolution of plant form. Oxford: Wiley-Blackwell.
Doyle JA, Donoghue MJ. 1986. Seed plant phylogeny and the origin of angiosperms—an experimental cladistic approach. Botanical Review 52: 321–431.
Duff RJ, Villarreal JC, Cargill DC, Renzaglia KS. 2007. Progress and challenges toward developing a phylogeny and classification of the hornworts. The Bryologist 110: 214–243.
Edwards D, Duckett JG, Richardson JB. 1995. Hepatic characters in the earliest land plants. Nature 374: 635–636.
Egel R, Penny D. 2007. On the origin of meiosis in eukaryotic evolution: Coevolution of meiosis and mitosis from feeble beginnings. In: Egel R, Lankenau D-H eds. Recombination and meiosis. Berlin: Springer-Verlag. 249–288.

Feng S, Jacobsen SE, Reik W. 2010. Epigenetic reprogramming in plant and animal development. Science 330: 622–627.

Finet C, Timme RE, Delwiche CF, Marletaz F. 2010. Multigene phylogeny of the green lineage reveals the origin and diversification of land plants. Current Biology 20: 2217–2222.

Floyd GL, Stewart KD, Mattox KR. 1972. Cellular organization, mitosis, and cytokinesis in the ulotrichalean alga, *Klebsormidium*. Journal of Phycology 8: 176–184.

Forrest LL, Davis EC, Long DG, Crandall-Stotler BJ, Clark A, Hollingsworth ML. 2006. Unraveling the evolutionary history of the liverworts (Marchantiophyta): Multiple taxa, genomes and analyses. The Bryologist 109: 303–334.

Fowke LC, Pickett-Heaps JD. 1969. Cell division in *Spirogyra*. II. Cytokinesis. Journal of Phycology 5: 273–281.

Frada M, Probert I, Allen MJ, Wilson WH, de Vargas C. 2008. The "Cheshire Cat" escape strategy of the coccolithophore *Emiliania huxleyi* in response to viral infection. Proceedings of the National Academy of Sciences USA 105: 15944–15949.

Frey W, Hofmann M, Hilger HH. 2001. The gametophyte–sporophyte junction: Unequivocal hints for two evolutionary lines of archegoniate land plants. Flora 196: 431–445.

Friedman WE. 1995. Organismal duplication, inclusive fitness theory, and altruism: Understanding the evolution of endosperm and the angiosperm reproductive syndrome. Proceedings of the National Academy of Sciences USA 92: 3913–3917.

Friedman WE, Williams JH. 2003. Modularity of the angiosperm female gametophyte and its bearing on the early evolution of endosperm in flowering plants. Evolution 57: 216–230.

Gensel PG. 2008. The earliest land plants. Annual Review of Ecology, Evolution, and Systematics 39: 459–477.

Gerrienne P, Dilcher DL, Bergamaschi S, Milagres I, Pereira E, Rodrigues MAC. 2006. An exceptional specimen of the early land plant *Cooksonia paranensis*, and a hypothesis on the life cycle of the earliest eutracheophytes. Review of Palaeobotany and Palynology 142: 123–130.

Gerrienne P, Gonez P. 2011. The early evolution of life cycles in embryophytes: A focus on the fossil evidence of gametophyte/sporophyte size and morphological complexity. Journal of Systematics and Evolution 49: 1–16.

Gifford EM, Foster AS. 1989. Morphology and evolution of vascular plants. New York: W. H. Freeman.

Goffinet B, Cox CJ, Shaw AJ, Hedderson TAJ. 2001. The bryophyta (mosses): Systematic and evolutionary inferences from an *rps4* gene (cpDNA) phylogeny. Annals of Botany 87: 191–208.

Goremykin VV, Hellwig FH. 2005. Evidence for the most basal split in land plants dividing bryophyte and tracheophyte lineages. Plant Systematics and Evolution 254: 93–103.

Graham LE. 1993. Origin of land plants. New York: John Wiley & Sons.

Graham LE, Cook ME, Busse JS. 2000. The origin of plants: Body plan changes contributing to a major evolutionary radiation. Proceedings of the National Academy of Sciences USA 97: 4535–4540.

Graham LE, Delwiche CF, Mishler BD. 1991. Phylogenetic connections between the 'green algae' and the 'bryophytes'. Advances in Bryology 4: 213–244.

Graham LE, Wilcox LW. 2000a. Algae. Upper Saddle River: Prentice Hall.

Graham LKE, Wilcox LW. 2000b. The origin of alternation of generations in land plants: A focus on matrotrophy and hexose transport. Philosophical Transactions of the Royal Society of London Series B: Biological Sciences 355: 757–767.

Grant V. 1963. The origin of adaptations. New York: Columbia University Press.

Gray J. 1985. The microfossil record of early land plants: Advances in understanding of early terrestrialization, 1970–1984. Philosophical Transactions of the Royal Society of London Series B: Biological Sciences 309: 167–195.

Gray J. 1993. Major Paleozoic land plant evolutionary bioevents. Palaeogeography Palaeoclimatology Palaeoecology 104: 153–169.

Gross J, Bhattacharya D. 2010. Uniting sex and eukaryote origins in an emerging oxygenic world. Biology Direct 5: 53.

Groth-Malonek M, Pruchner D, Grewe F, Knoop V. 2005. Ancestors of trans-splicing mitochondrial introns support serial sister group relationships of hornworts and mosses with vascular plants. Molecular Biology and Evolution 22: 117–125.

Guignard ML. 1885. Nouvelles recherches sur le noyau cellulaire. Annales de Sciences Naturelles, Botanique 20: 310–372.

Hackett JD, Yoon HS, Li S, Reyes-Prieto A, Rummele SE, Bhattacharya D. 2007. Phylogenomic analysis supports the monophyly of cryptophytes and haptophytes and the association of *Rhizaria* with Chromalveolates. Molecular Biology and Evolution 24: 1702–1713.

Haig D. 2010. What do we know about charophyte (Streptophyta) life cycles? Journal of Phycology 46: 860–867.

Haig D, Westoby M. 1989. Parent-specific gene expression and the triploid endosperm. American Naturalist 134: 147–155.

He-Nygren X, Ahonen I, Juslen A, Glenny D, Piippo S. 2004. Phylogeny of liverworts: Beyond a leaf and a thallus. In: Goffinet B, Hollowell V, Magill R eds. Molecular systematics of bryophytes. St. Louis: Missouri Botanical Garden Press. 87–118.

Heath TA, Hedtke SM, Hillis DM. 2008. Taxon sampling and the accuracy of phylogenetic analyses. Journal of Systematics and Evolution 46: 239–257.

Hébant C. 1977. The conducting tissues of bryophytes. Vaduz: J. Cramer.

Heinrichs J, Gradstein SR, Wilson R, Schneider H. 2005. Towards a natural classification of liverworts (Marchantiophyta) based on the chloroplast gene *rbcL*. Cryptogamie Bryologie 26: 131–150.

Hennig W. 1966. Phylogenetic systematics. Urbana: University of Illinois Press.

Hofmeister W. 1851. Vergleichende Untersuchungen der Keimung, Entfaltung und Fruchbildiung hoeherer Kryptogamen (Moose, Farne, Equisetaceen, Rhizocarpeen und Lycopodiaceen) und der Samenbildiung der Coniferen. Lepizig: F. Hofmeister.

Hofmeister W. 1862. On the germination, development, and fructification of the higher Cryptogamia, and on the fructification of the Coniferae. Published for the Ray Society. London: Robert Hardwicke.

Houdan A, Billard C, Marie D, Not F, Saez AG, Young JR, Probert I. 2004. Holococcolithophore-heterococcolithophore (Haptophyta) life cycles: Flow

cytometric analysis of relative ploidy levels. Systematics and Biodiversity 1: 453–465.

Iino Y, Yamamoto M. 1985. Negative control for the initiation of meiosis in *Schizosaccharomyces pombe*. Proceedings of the National Academy of Sciences USA 82: 2447–2451.

James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, Cox CJ, Celio G, Gueidan C, Fraker E, Miadlikowska J, Lumbsch HT, Rauhut A, Reeb V, Arnold AE, Amtoft A, Stajich JE, Hosaka K, Sung GH, Johnson D, O'Rourke B, Crockett M, Binder M, Curtis JM, Slot JC, Wang Z, Wilson AW, Schussler A, Longcore JE, O'Donnell K, Mozley-Standridge S, Porter D, Letcher PM, Powell MJ, Taylor JW, White MM, Griffith GW, Davies DR, Humber RA, Morton JB, Sugiyama J, Rossman AY, Rogers JD, Hewitt D, Hansen K, Hambleton S, Shoemaker RA, Kohlmeyer J, Volkmann-Kohlmeyer B, Spotts RA, Serdani M, Crous PW, Hughes KW, Matsuura K, Langer E, Langer G, Untereiner WA, Lucking R, Budel B, Geiser DM, Aptroot A, Diederich P, Schmitt I, Schultz M, Yahr R, Hibbett DS, Lutzoni F, McLaughlin DJ, Spatafora JW, Vilgalys R. 2006. Reconstructing the early evolution of Fungi using a six-gene phylogeny. Nature 443: 818–822.

Jeffares DC, Phillips MJ, Moore S, Veit B. 2004. A description of the Mei2-like protein family; structure, phylogenetic distribution and biological context. Development Genes and Evolution 214: 149–158.

Jeffrey C. 1967. The origin and differentiation of the archegoniate land plants: A second contribution. Kew Bulletin 21: 335–349.

Jeffrey C. 1982. Kingdoms, codes and classification. Kew Bulletin 37: 403–416.

Jia Y, Wu P-C, Wang M-Z, He S. 2003. Takakiopsida, a unique taxon of bryophytes. Acta Phytotaxonomica Sinica 41: 350–361.

Johri BM, Ambegaokar KB, Srivastava PS. 1992. Comparative embryology of angiosperms. Berlin: Springer-Verlag.

Jones JD, Dangl JL. 2006. The plant immune system. Nature 444: 323–329.

Karol KG, McCourt RM, Cimino MT, Delwiche CF. 2001. The closest living relatives of land plants. Science 294: 2351–2353.

Kato M, Akiyama H. 2005. Interpolation hypothesis for origin of the vegetative sporophyte of land plants. Taxon 54: 443–450.

Kaufman AJ, Xiao SH. 2003. High CO_2 levels in the Proterozoic atmosphere estimated from analyses of individual microfossils. Nature 425: 279–282.

Kaur J, Sebastian J, Siddiqi I. 2006. The *Arabidopsis-mei2*-like genes play a role in meiosis and vegetative growth in *Arabidopsis*. Plant Cell 18: 545–559.

Kawachi M, Inouye I, Honda D, O'Kelly CJ, Bailey JC, Bidigare RR, Andersen RA. 2002. The Pinguiophyceae classis nova, a new class of photosynthetic stramenopiles whose members produce large amounts of omega-3 fatty acids. Phycological Research 50: 31–47.

Keddy PA. 1981. Why gametophytes and sporophytes are different: Form and function in a terrestrial environment. American Naturalist 118: 452–454.

Keeling PJ, Burger G, Durnford DG, Lang BF, Lee RW, Pearlman RE, Roger AJ, Gray MW. 2005. The tree of eukaryotes. Trends in Ecology and Evolution 20: 670–676.

Kelch DG, Driskell A, Mishler BD. 2004. Inferring phylogeny using genomic characters: A case study using land plant plastomes. In: Goffinet B, Hollowell V, Magill R eds. Molecular systematics of bryophytes. St. Louis: Missouri Botanical Garden Press. 3–11.

Kenrick P. 1994. Alternation of generations in land plants: New phylogenetic and palaeobotanical evidence. Biological Reviews 69: 293–330.

Kenrick P. 2000. The relationships of vascular plants. Philosophical Transactions of the Royal Society of London Series B: Biological Sciences 355: 847–855.

Kenrick P, Crane PR. 1997. The origin and early diversification of land plants: A cladistic study. Washington: Smithsonian Institution Press.

Knoll AH. 2003. Life on a young planet. Princeton: Princeton University Press.

Knoll AH, Javaux EJ, Hewitt D, Cohen P. 2006. Eukaryotic organisms in Proterozoic oceans. Philosophical Transactions of the Royal Society of London Series B: Biological Sciences 361: 1023–1038.

Kondrashov AS. 1988. Deleterious mutations and the evolution of sexual reproduction. Nature 336: 435–440.

Kondrashov AS. 1997. Evolutionary genetics of life cycles. Annual Review of Ecology and Systematics 28: 391–435.

Kondrashov AS, Crow JF. 1991. Haploidy or diploidy: Which is better? Nature 351: 314–315.

Kramer KU, Green PS. 1990. Pteridophytes and gymnosperms. Berlin: Springer-Verlag.

Kreulen DJW. 1972. Spore output of moss capsules in relationship to ontogeny of archesporial tissue. Journal of Bryology 7: 61–74.

Laird CD, McConaughy BL, McCarthy BJ. 1969. Rates of fixation of nucleotide substitutions in evolution. Nature 224: 149–154.

Lazarus R, Vercelli D, Palmer LJ, Klimecki WJ, Silverman EK, Richter B, Riva A, Ramoni M, Martinez FD, Weiss ST, Kwiatkowski DJ. 2002. Single nucleotide polymorphisms in innate immunity genes: Abundant variation and potential role in complex human disease. Immunological Reviews 190: 9–25.

Leitch IJ, Soltis DE, Soltis PS, Bennett MD. 2005. Evolution of DNA amounts across land plants (Embryophyta). Annals of Botany 95: 207–217.

Lemieux C, Otis C, Turmel M. 2007. A clade uniting the green algae *Mesostigma viride* and *Chlorokybus atmophyticus* represents the deepest branch of the Streptophyta in chloroplast genome-based phylogenies. BMC Biology 5: 2.

Lewis J, Wolpert L. 1979. Diploidy, evolution and sex. Journal of Theoretical Biology 78: 425–438.

Lewis JWM. 1985. Nutrient scarcity as an evolutionary cause of haploidy. American Naturalist 125: 692–701.

Lewis LA, McCourt RM. 2004. Green algae and the origin of land plants. American Journal of Botany 91: 1535–1556.

Li W-H. 1997. Molecular evolution. Sunderland: Sinauer Associates.

Li W-H, Yi S, Makova K. 2002. Male-driven evolution. Current Opinion in Genetics and Development 12: 650–656.

Lindeman RL. 1942. The trophic-dynamic aspect of ecology. Ecology 23: 399–417.

Longton RE, Schuster RM. 1983. Reproductive biology. In: Schuster RM ed. New manual of bryology. Nichinan: The Hattori Botanical Laboratory. 386–462.

Losos JB. 2008. Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. Ecology Letters 11: 995–1007.

Mable BK, Otto SP. 1998. The evolution of life cycles with haploid and diploid phases. Bioessays 20: 453–462.

MacArthur RH, Wilson EO. 1967. The theory of island biogeography. Princeton: Princeton University Press.

Manhart JR, Palmer JD. 1990. The gain of two chloroplast transfer-RNA introns marks the green algal ancestors of land plants. Nature 345: 268–270.

Marchant HJ, Pickett-Heaps JD. 1973. Mitosis and cytokinesis in *Coleochaete scutata*. Journal of Phycology 9: 461–471.

Maynard Smith J, Szathmary E. 1995. The major transitions in evolution. Oxford: Oxford University Press.

McManus HA, Qiu Y-L. 2008. Life cycles in major lineages of photosynthetic eukaryotes, with a special reference to the origin of land plants. Fieldiana 47: 17–33.

McManus HA, Qiu Y-L. 2010. On alternations of generations in embryophytic plants. In: Long MY, Gu HY, Zhou ZH eds. Darwin's heritage today: Proceedings of the Darwin 200 Beijing international conference. Beijing: Higher Education Press. 190–200.

Melkonian M. 1989. Flagellar apparatus ultrastructure in *Mesostigma viride* (Prasinophyceae). Plant Systematics and Evolution 164: 93–122.

Messaoudi I, Guevara Patiño JA, Dyall R, LeMaoult J, Nikolich-Zugich J. 2002. Direct link between mhc polymorphism, T cell avidity, and diversity in immune defense. Science 298: 1797–1800.

Mishler BD, Churchill SP. 1984. A cladistic approach to the phylogeny of the bryophytes. Brittonia 36: 406–424.

Mishler BD, Churchill SP. 1985. Transition to a land flora: Phylogenetic relationships of the green algae and bryophytes. Cladistics 1: 305–328.

Miyata T, Hayashida H, Kuma K, Mitsuyasu K, Yasunaga T. 1987. Male-driven molecular evolution: A model and nucleotide sequence analysis. Cold Spring Harbor Symposia on Quantitative Biology 52: 863–867.

Mora CI, Driese SG, Seager PG. 1991. Carbon dioxide in the Paleozoic atmosphere: Evidence from carbon-isotope compositions of pedogenic carbonate. Geology 19: 1017–1020.

Morgan TH. 1932. The scientific basis of evolution. New York: Norton.

Nei M. 2007. The new mutation theory of phenotypic evolution. Proceedings of the National Academy of Sciences USA 104: 12235–12242.

Nishiyama T, Wolf PG, Kugita M, Sinclair RB, Sugita M, Sugiura C, Wakasugi T, Yamada K, Yoshinaga K, Yamaguchi K, Ueda K, Hasebe M. 2004. Chloroplast phylogeny indicates that bryophytes are monophyletic. Molecular Biology and Evolution 21: 1813–1819.

Nuismer SL, Otto SP. 2004. Host–parasite interactions and the evolution of ploidy. Proceedings of the National Academy of Sciences USA 101: 11036–11039.

Odum EP. 1969. The strategy of ecosystem development. Science 164: 262–270.

Odum EP, Barrett GW. 2005. Fundamentals of ecology. Belmont: Thomson Brooks/Cole.

Odum HT. 1988. Self-organization, transformity, and information. Science 242: 1132–1139.

Ohno S. 1970. Evolution by gene duplication. New York: Springer-Verlag.

Olmedo-Monfil V, Duran-Figueroa N, Arteaga-Vazquez M, Demesa-Arevalo E, Autran D, Grimanelli D, Slotkin RK, Martienssen RA, Vielle-Calzada J-P. 2010. Control of female gamete formation by a small RNA pathway in *Arabidopsis*. Nature 464: 628–632.

Orr HA. 1995. Somatic mutation favors the evolution of diploidy. Genetics 139: 1441–1447.

Otto SP, Marks JC. 1996. Mating systems and the evolutionary transition between haploidy and diploidy. Biological Journal of the Linnean Society 57: 197–218.

Overton E. 1893. On the reduction of the chromosomes in the nuclei of plants. Annals of Botany 7: 139–143.

Paquin C, Adams J. 1983. Frequency of fixation of adaptive mutations is higher in evolving diploid than haploid yeast populations. Nature 302: 495–500.

Parenti LR. 1980. A phylogenetic analysis of the land plants. Biological Journal of the Linnean Society 13: 225–242.

Parfrey LW, Grant J, Tekle YI, Lasek-Nesselquist E, Morrison HG, Sogin ML, Patterson DJ, Katz LA. 2010. Broadly sampled multigene analyses yield a well-resolved eukaryotic tree of life. Systematic Biology 59: 518–533.

Parkinson BM. 1987. Tapetal organization during sporogenesis in *Psilotum nudum*. Annals of Botany 60: 353–360.

Payne JL, Boyer AG, Brown JH, Finnegan S, Kowalewski M, Krause Jr, RA, Lyons SK, McClain CR, McShea DW, Novack-Gottshall PM, Smith FA, Stempien JA, Wang SC. 2009. Two-phase increase in the maximum size of life over 3.5 billion years reflects biological innovation and environmental opportunity. Proceedings of the National Academy of Sciences USA 106: 24–27.

Perrot V, Richerd S, Valero M. 1991. Transition from haploidy to diploidy. Nature 351: 315–317.

Pickett-Heaps JD. 1967. Ultrastructure and differentiation in *Chara* sp. II. Mitosis. Australian Journal of Biological Science 20: 883–894.

Pickett-Heaps JD. 1972. Cell division in *Klebsormidium subtilissimum* (formerly *Ulothrix subtilissima*), and its possible phylogenetic significance. Cytobios 6: 167–183.

Pickett-Heaps JD. 1975. Green algae: Structure, reproduction and evolution in selected genera. Sunderland: Sinauer Associates.

Pringsheim N. 1878. Ueber Sprossung der Moosfruechte und den Generationswechel der Thallophyten. Jahrbuecher fuer wissenschaftliche Botanik 11: 1–46.

Proskauer J. 1960. Studies on Anthocerotales VI. Phytomorphology 10: 1–19.

Pryer KM, Schneider H, Smith AR, Cranfill R, Wolf PG, Hunt JS, Sipes SD. 2001. Horsetails and ferns are a monophyletic group and the closest living relatives to seed plants. Nature 409: 618–622.

Qiu Y-L. 2008. Phylogeny and evolution of charophytic algae and land plants. Journal of Systematics and Evolution 46: 287–306.

Qiu Y-L, Cho YR, Cox JC, Palmer JD. 1998. The gain of three mitochondrial introns identifies liverworts as the earliest land plants. Nature 394: 671–674.

Qiu Y-L, Li L, Wang B, Hendry TA, Li R, Brown JW, Liu Y, Hudson GT, Chen Z. 2010. Angiosperm phylogeny inferred from sequences of four mitochondrial genes. Journal of Systematics and Evolution 48: 391–425.

Qiu Y-L, Li LB, Wang B, Chen ZD, Dombrovska O, Lee J, Kent L, Li RQ, Jobson RW, Hendry TA, Taylor DW, Testa CM, Ambros M. 2007. A nonflowering land plant phylogeny inferred from nucleotide sequences of seven chloroplast, mitochondrial, and nuclear genes. International Journal of Plant Sciences 168: 691–708.

Qiu Y-L, Li LB, Wang B, Chen ZD, Knoop V, Groth-Malonek M, Dombrovska O, Lee J, Kent L, Rest J, Estabrook GF, Hendry TA, Taylor DW, Testa CM, Ambros M, Crandall-Stotler B, Duff RJ, Stech M, Frey W, Quandt D, Davis CC. 2006. The deepest divergences in land plants inferred from phylogenomic evidence. Proceedings of the National Academy of Sciences USA 103: 15511–15516.

Raubeson LA, Jansen RK. 1992. Chloroplast DNA evidence on the ancient evolutionary split in vascular land plants. Science 255: 1697–1699.

Raven PH, Evert RF, Eichhorn SE. 2005. Biology of plants. New York: W. H. Freeman.

Remy W, Gensel PG, Hass H. 1993. The gametophyte generation of some Early Devonian land plants. International Journal of Plant Sciences 154: 35–58.

Renzaglia KS, Duff RJ, Nickrent DL, Garbary DJ. 2000. Vegetative and reproductive innovations of early land plants: Implications for a unified phylogeny. Philosophical Transactions of the Royal Society of London Series B: Biological Sciences 355: 769–793.

Renzaglia KS, McFarland KD, Smith DK. 1997. Anatomy and ultrastructure of the sporophyte of *Takakia ceratophylla* (Bryophyta). American Journal of Botany 84: 1337–1350.

Rinard RG. 1981. The problem of the organic individual: Ernst Haeckel and the development of the biogenetic law. Journal of the History of Biology 14: 249–275.

Rodriguez-Ezpeleta N, Brinkmann H, Roure B, Lartillot N, Lang BF, Philippe H. 2007. Detecting and overcoming systematic errors in genome-scale phylogenies. Systematic Biology 56: 389–399.

Rogers CE, Domozych DS, Stewart KD, Mattox KR. 1981. The flagellar apparatus of *Mesostigma viride* (Prasinophyceae): Multilayered structures in a scaly green flagellate. Plant Systematics and Evolution 138: 247–258.

Rogers CE, Mattox KR, Stewart KD. 1980. The zoospores of *Chlorokybus atmophyticus*, a charophyte with sarcinoid growth habit. American Journal of Botany 67: 774–783.

Samigullin TK, Yacentyuk SP, Degtyaryeva GV, Valiehoroman KM, Bobrova VK, Capesius I, Martin WM, Troitsky AV, Filin VR, Antonov AS. 2002. Paraphyly of bryophytes and close relationship of hornworts and vascular plants inferred from analysis of chloroplast rDNA ITS (cpITS) sequences. Arctoa 11: 31–43.

Sars M. 1837. Beitrag zur Entwicklungsgeschichte der Mollusken und Zoophyten. Archiv fuer Naturgeschichte 3: 402–407.

Sars M. 1840. Beitrag zur Entwicklungsgeschichte der Mollusken und Zoophyten. Archiv fuer Naturgeschichte 6: 196–219.

Saunders GW, Hommersand MH. 2004. Assessing red algal supraordinal diversity and taxonomy in the context of contemporary systematic data. American Journal of Botany 91: 1494–1507.

Schulz C, Little DP, Stevenson DW, Bauer D, Moloney C, Stutzel T. 2010. An overview of the morphology, anatomy, and life cycle of a new model species: The lycophyte *Selaginella apoda* (L.) Spring. International Journal of Plant Sciences 171: 693–712.

Schuster RM. 1966. The Hepaticae and Anthocerotae of North America, Vol. I. New York: Columbia University Press.

Schuster RM. 1981. Paleoecology, origin, distribution through time, and evolution of Hepaticae and Anthocerotae. In: Niklas KJ ed. Paleobotany, paleoecology, and evolution. New York: Praeger.

Schuster RM. 1992a. The Hepaticae and Anthocerotae of North America, Vol. V. Chicago: Field Museum of Natural History.

Schuster RM. 1992b. The Hepaticae and Anthocerotae of North America, Vol. VI. Chicago: Field Museum of Natural History.

Schwartzman DW, Volk T. 1989. Biotic enhancement of weathering and the habitability of Earth. Nature 340: 457–460.

Searles RB. 1980. The strategy of the red algal life history. American Naturalist 115: 113–120.

Simpson AGB, Inagaki Y, Roger AJ. 2006. Comprehensive multigene phylogenies of excavate protists reveal the evolutionary positions of "primitive" eukaryotes. Molecular Biology and Evolution 23: 615–625.

Singh H. 1978. Embryology of gymnosperms. Berlin: Gebrueder Borntraeger.

Slotkin RK, Vaughn M, Borges F, Tanurdzic M, Becker JD, Feijo JA, Martienssen RA. 2009. Epigenetic reprogramming and small RNA silencing of transposable elements in pollen. Cell 136: 461–472.

Smith FG. 1907. Morphology of the trunk and development of the microsporangium of cycads. Botanical Gazette 43: 187–204.

Smith GM. 1955. Cryptogamic botany, Vol. I. Bryophytes and pteridophytes. New York: McGraw-Hill.

Smith RW. 1900. The structure and development of the sporophylls and sporangia of *Isoetes*. Botanical Gazette 29: 225–258, 323–346.

Smith SA, Donoghue MJ. 2008. Rates of molecular evolution are linked to life history in flowering plants. Science 322: 86–89.

Soltis DE, Smith SA, Cellinese N, Wurdack KJ, Tank DC, Brockington SF, Refulio-Rodriguez NF, Walker JB, Moore MJ, Carlsward BS, Bell CD, Latvis M, Crawley S, Black C, Diouf D, Xi Z, Gitzendanner MA, Sytsma KJ, Qiu Y-L, Hilu KW, Davis CC, Sanderson MJ, Olmstead RG, Judd WS, Donoghue MJ, Soltis PS. 2011. Angiosperm phylogeny: 17 genes, 640 taxa. American Journal of Botany 98: 704–730.

Stark LR. 2002. Phenology and its repercussions on the reproductive ecology of mosses. Bryologist 105: 204–218.

Stebbins GL, Hill GJC. 1980. Did multicellular plants invade the land? American Naturalist 115: 342–353.

Steemans P, Le Hérissé A, Melvin J, Miller MA, Paris F, Verniers J, Wellman CH. 2009. Origin and radiation of the earliest vascular land plants. Science 324: 353.

Steenstrup JJS. 1842. Om Forplantning og Udvikling gjennem vexlende Generationsraekker, en saeregen form for Opfostringen ide lavere Dyreklasser. Copenhagen: Bianco Lunos.
Steenstrup JJS. 1845. On the alternation of generations; or, The propagation and development of animals through alternate generations: A peculiar form of fostering the young in the lower classes of animals. London: The Ray Society.
Stewart KD, Mattox KR. 1975. Comparative cytology, evolution and classification of the green algae with some consideration of the origin of other organisms with chlorophylls a and b. Botanical Review 41: 104–135.
Stewart WDP, Rodgers GA. 1977. The cyanophyte–hepatic symbiosis. II. Nitrogen-fixation and the interchange of the nitrogen and carbon. New Phytologist 78: 459–471.
Stewart-Cox JA, Britton NF, Mogie M. 2004. Endosperm triploidy has a selective advantage during ongoing parental conflict by imprinting. Proceedings of the Royal Society of London Series B: Biological Sciences 271: 1737–1743.
Strasburger E. 1894. The periodic reduction of the number of the chromosomes in the life-history of living organisms. Annals of Botany 8: 281–316.
Strother PK, Al-Hajri S, Traverse A. 1996. New evidence for land plants from the lower Middle Ordovician of Saudia Arabia. Geology 24: 55–58.
Strother PK, Wood GD, Taylor WA, Beck JH. 2004. Middle Cambrian cryptospores and the origin of land plants. Memoirs of the Association of Australasian Palaeontologists 29: 99–113.
Svedelius N. 1921. Einige Bemerkungen ueber Generationswechsel und Reduktionsteilung. Berichte der Deutschen Botanischen Gesellschaft 39: 178–187.
Svedelius N. 1927. Alternation of generations in relation to reduction division. Botanical Gazette 83: 362–384.
Takayama S, Isogai A. 2005. Self-incompatibility in plants. Annual Review of Plant Biology 56: 467–489.
Tanahashi T, Sumikawa N, Kato M, Hasebe M. 2005. Diversification of gene function: Homologs of the fioral regulator FLO/LFY control the first zygotic cell division in the moss *Physcomitrella patens*. Development 132: 1727–1736.
Tanurdzic M, Banks JA. 2004. Sex-determining mechanisms in land plants. Plant Cell 16: S61–S71.
Taylor TN, Kerp H, Hass H. 2005. Life history biology of early land plants: Deciphering the gametophyte phase. Proceedings of the National Academy of Sciences USA 102: 5892–5897.
Taylor WA. 1995. Spores in earliest land plants. Nature 373: 391–392.
Taylor WA. 2003. Ultrastructure of selected Silurian trilete spores and the putative Ordovician trilete spore *Virgatasporites*. Review of Palaeobotany and Palynology 126: 211–223.
Theissen G, Becker A, Di Rosa A, Kanno A, Kim JT, Munster T, Winter KU, Saedler H. 2000. A short history of MADS-box genes in plants. Plant Molecular Biology 42: 115–149.
Tryon AF, Lugardon B. 1991. Spores of the pteridophyta. New York: Springer.
Turmel M, Pombert JF, Charlebois P, Otis C, Lemieux C. 2007. The green algal ancestry of land plants as revealed by the chloroplast genome. International Journal of Plant Sciences 168: 679–689.
Van Beneden E. 1883. Recherches sur la maturation de l'oeuf et la fécondation. *Ascaris megalocephala*. Archives de Biologie 4: 265–640.
van den Hoek C, Mann DG, Jahns HM. 1995. Algae: An introduction to phycology. Cambridge: Cambridge University Press.
Veit B, Briggs SP, Schmidt RJ, Yanofsky MF, Hake S. 1998. Regulation of leaf initiation by the *terminal ear 1* gene of maize. Nature 393: 166–168.
Walter KS. 1983. Orchidaceae. In: Janzen DH ed. Costa Rican natural history. Chicago: The University of Chicago Press. 282–292.
Wang B, Yeun LH, Xue J-Y, Liu Y, Ané JM, Qiu Y-L. 2010. Presence of three mycorrhizal genes in the common ancestor of land plants suggests a key role of mycorrhizas in the colonization of land by plants. New Phytologist 186: 514–525.
Watanabe Y, Yamamoto M. 1994. *S. pombe mei2*$^{+}$ encodes an RNA-binding protein essential for premeiotic DNA synthesis and meiosis I, which cooperates with a novel RNA species meiRNA. Cell 78: 487–498.
Wellman CH, Osterloff PL, Mohiuddin U. 2003. Fragments of the earliest land plants. Nature 425: 282–285.
Wiens JJ. 2004. Speciation and ecology revisited: Phylogenetic niche conservatism and the origin of species. Evolution 58: 193–197.
Wilson MA, Makova KD. 2009. Genomic analyses of sex chromosome evolution. Annual Review of Genomics and Human Genetics 10: 333–354.
Wodniok S, Brinkmann H, Glockner G, Heidel AJ, Philippe H, Melkonian M, Becker B. 2011. Origin of land plants: Do conjugating green algae hold the key? BMC Evolutionary Biology 11: 104.
Wolf PG, Karol KG, Mandoli DF, Kuehl J, Arumuganathan K, Ellis MW, Mishler BD, Kelch DG, Olmstead RG, Boore JL. 2005. The first complete chloroplast genome sequence of a lycophyte, *Huperzia lucidula* (Lycopodiaceae). Gene 350: 117–128.
Yamanouchi S. 1906a. The life history of *Polysiphonia violacea*. Contributions from the Hull Botanical Laboratory LXXXIII. Botanical Gazette 41: 425–433.
Yamanouchi S. 1906b. The life history of *Polysiphonia violacea*. Contributions from the Hull Botanical Laboratory LXXXVII. Botanical Gazette 42: 401–448.
Yoon HS, Hackett JD, Ciniglia C, Pinto G, Bhattacharya D. 2004. A molecular timeline for the origin of photosynthetic eukaryotes. Molecular Biology and Evolution 21: 809–818.
Zeyl C, Vanderford T, Carter M. 2003. An evolutionary advantage of haploidy in large yeast populations. Science 299: 555–558.
Zipfel C. 2008. Pattern-recognition receptors in plant innate immunity. Current Opinion in Immunology 20: 10–16.